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(54) **FACTOR VIII-FC CHIMERIC AND HYBRID POLYPEPTIDES, AND METHODS OF USE THEREOF**

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A61K 39/395	(2006.01)
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C07K 14/755	(2006.01)
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CPC ***A61K 38/37*** (2013.01); ***A61K 47/48284*** (2013.01); ***A61K 47/48415*** (2013.01); ***C07K 14/755*** (2013.01); *C07K 2319/30* (2013.01); ***C07K 16/46*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides methods of administering Factor VIII; methods of administering chimeric and hybrid polypeptides comprising Factor VIII; chimeric and hybrid polypeptides comprising Factor VIII; polynucleotides encoding such chimeric and hybrid polypeptides; cells comprising such polynucleotides; and methods of producing such chimeric and hybrid polypeptides using such cells.

31 Claims, 19 Drawing Sheets

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Figure 1

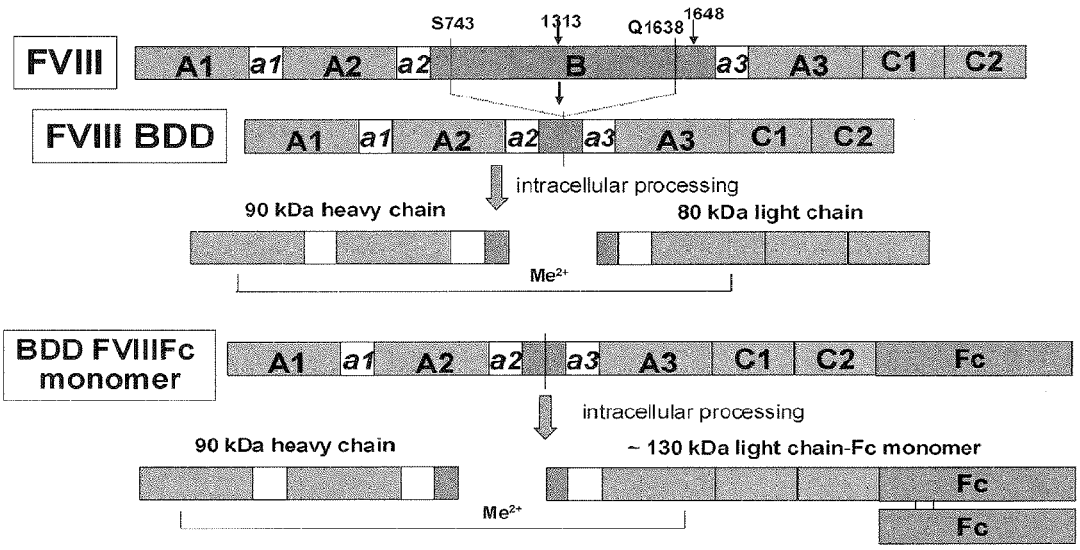

FVIII
S743
1313
Q1638
1648
A1
a1
A2
a2
B
a3
A3
C1
C2
FVIII BDD
intracellular processing
90 kDa heavy chain
80 kDa light chain
Me2+
BDD FVIIIFc monomer
Fc
intracellular processing
90 kDa heavy chain
~ 130 kDa light chain-Fc monomer
Me2+
Fc
Fc

Figure 2

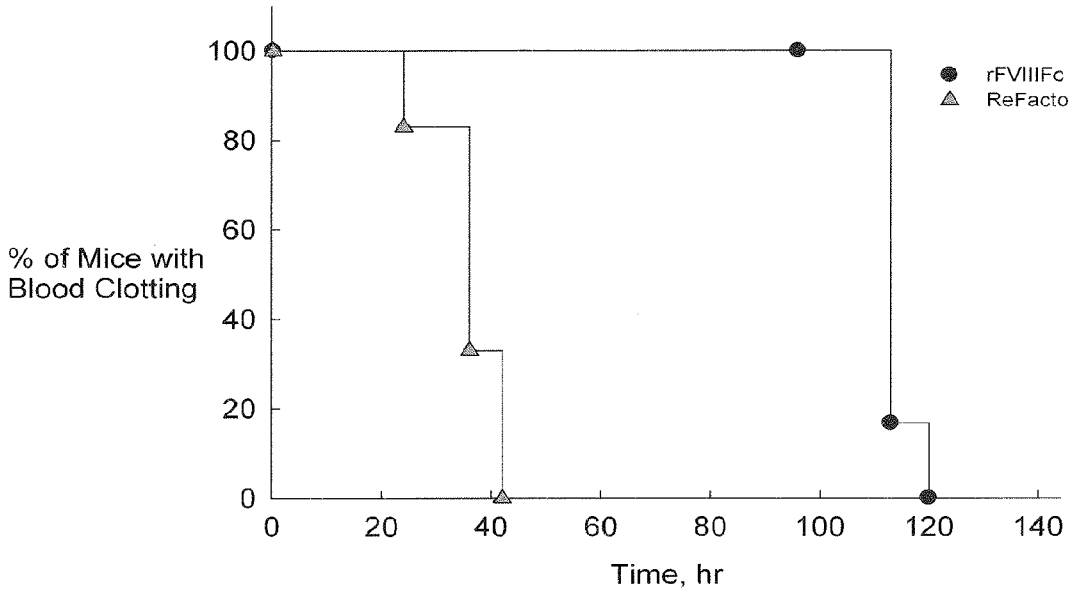

100
80
60
40
20
0
% of Mice with Blood Clotting
0
20
40
60
80
100
120
140
Time, hr
rFVIIIFc
ReFacto

Figure 3

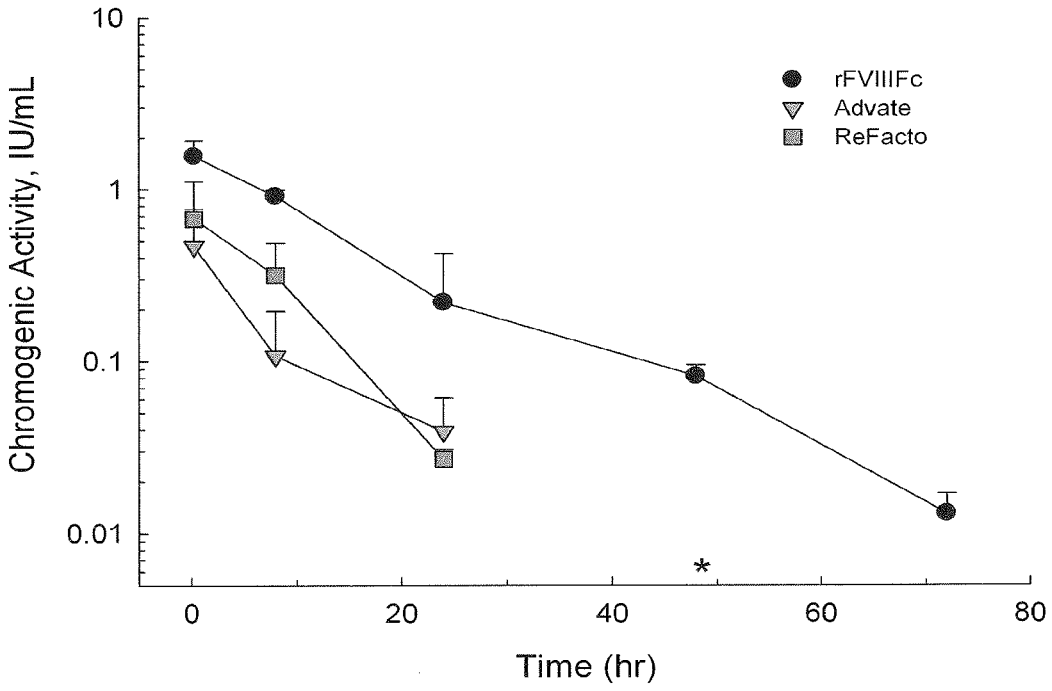

10
1
0.1
0.01
Chromogenic Activity, IU/mL
rFVIIIFc
Advate
ReFacto
*
0
20
40
60
80
Time (hr)

Figure 4A

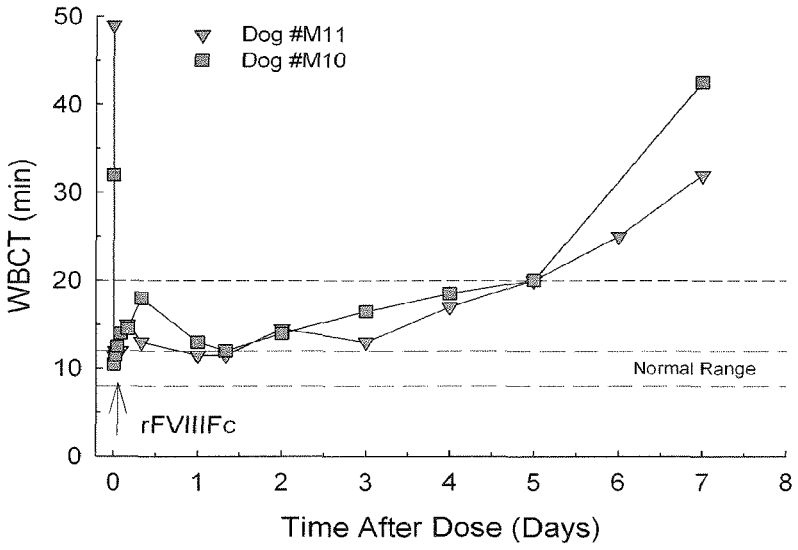

Dog #M11
Dog #M10
WBCT (min)
Time After Dose (Days)
Normal Range
rFVIIIFc
0
10
20
30
40
50
0
1
2
3
4
5
6
7
8

Figure 4B

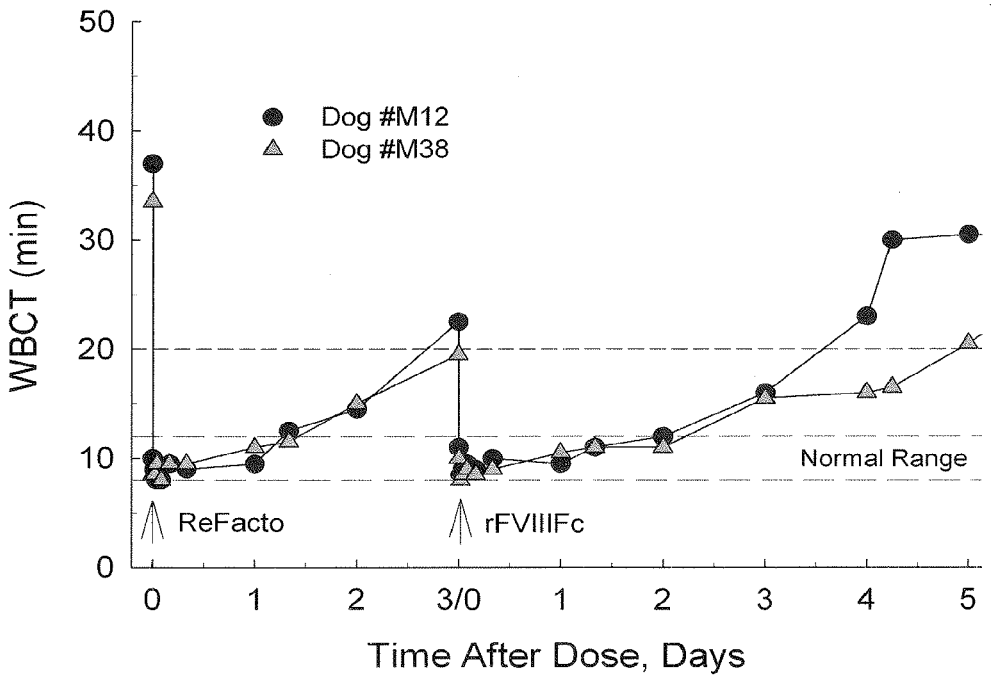

50
40
30
20
10
0
WBCT (min)
Dog #M12
Dog #M38
Normal Range
ReFacto
rFVIIIFc
0
1
2
3/0
1
2
3
4
5
Time After Dose, Days

Figure 5

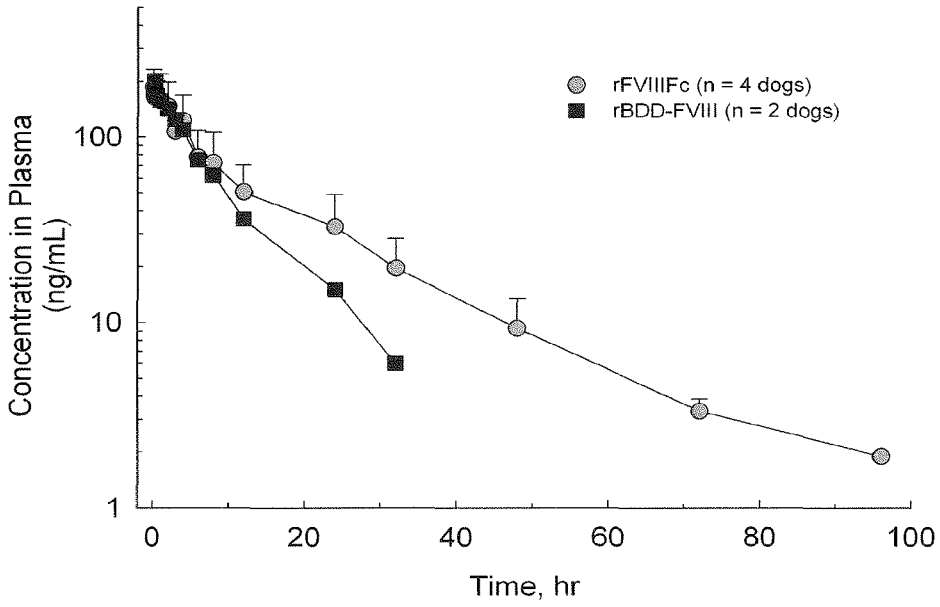

rFVIIIFc (n = 4 dogs)
rBDD-FVIII (n = 2 dogs)
Concentration in Plasma (ng/mL)
100
10
1
0
20
40
60
80
100
Time, hr

Figure 6

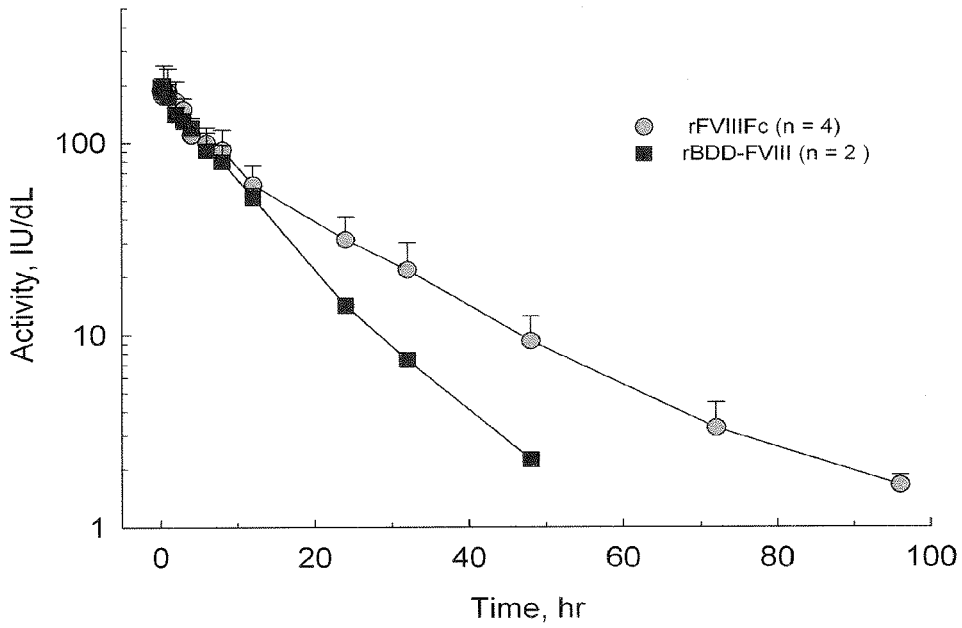

rFVIIIFc (n = 4)
rBDD-FVIII (n = 2)
Activity, IU/dL
100
10
1
0
20
40
60
80
100
Time, hr

Figure 7

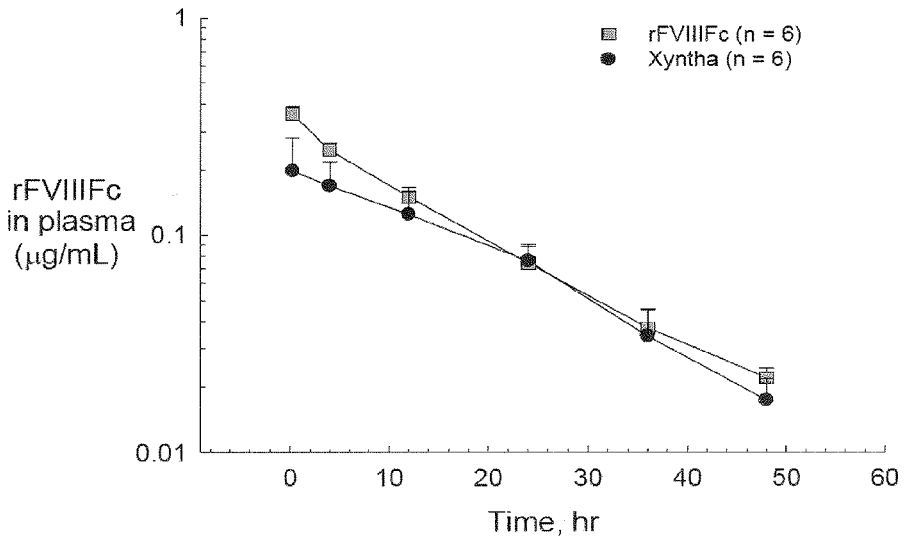

rFVIIIFc (n = 6)
Xyntha (n = 6)
rFVIIIFc in plasma (μg/mL)
1
0.1
0.01
0
10
20
30
40
50
60
Time, hr

Figure 8

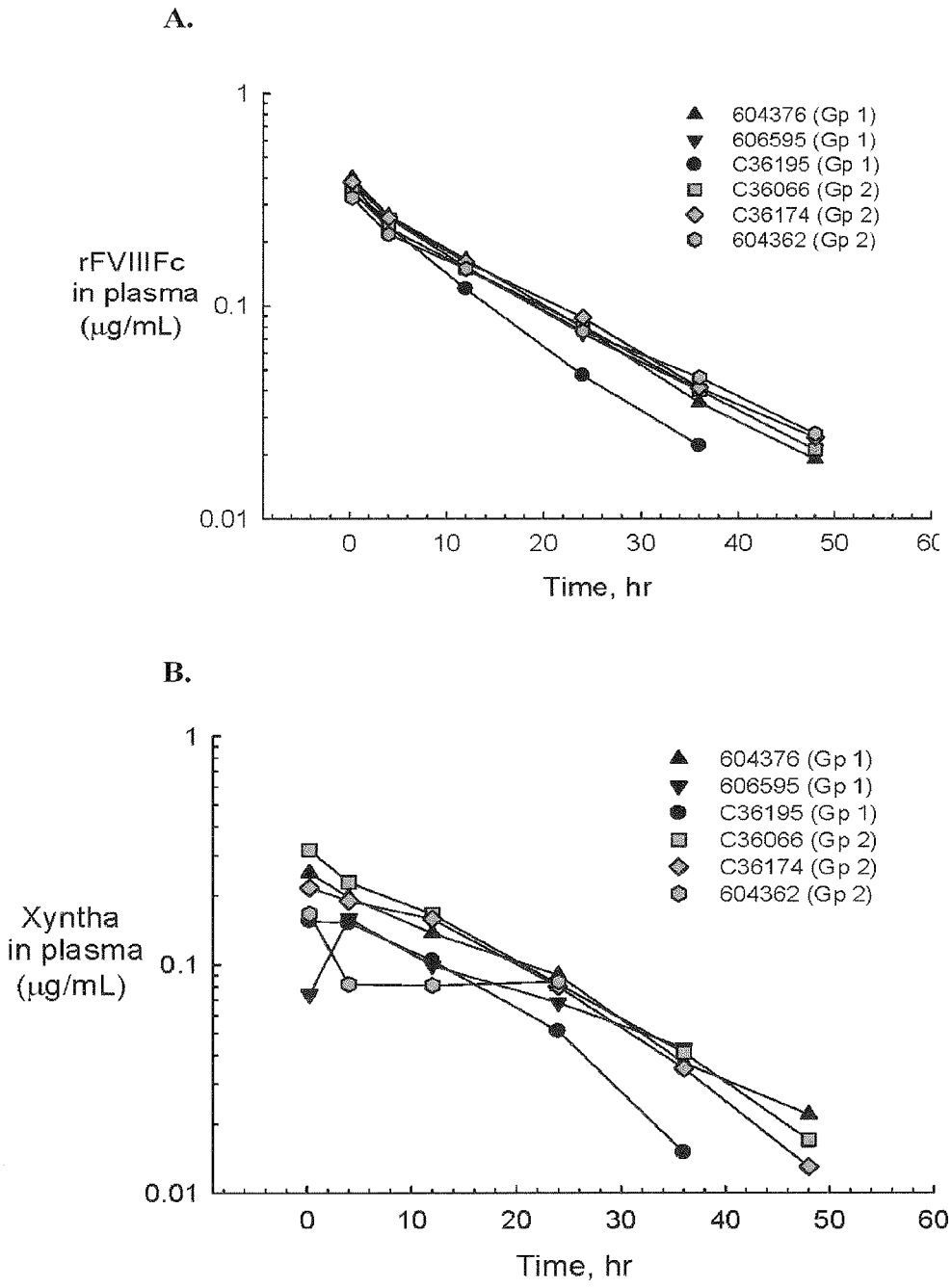

A.
rFVIIIFc in plasma (μg/mL)
1
0.1
0.01
0
10
20
30
40
50
60
Time, hr
604376 (Gp 1)
606595 (Gp 1)
C36195 (Gp 1)
C36066 (Gp 2)
C36174 (Gp 2)
604362 (Gp 2)
B.
Xyntha in plasma (μg/mL)
1
0.1
0.01
0
10
20
30
40
50
60
Time, hr
604376 (Gp 1)
606595 (Gp 1)
C36195 (Gp 1)
C36066 (Gp 2)
C36174 (Gp 2)
604362 (Gp 2)

Figure 9

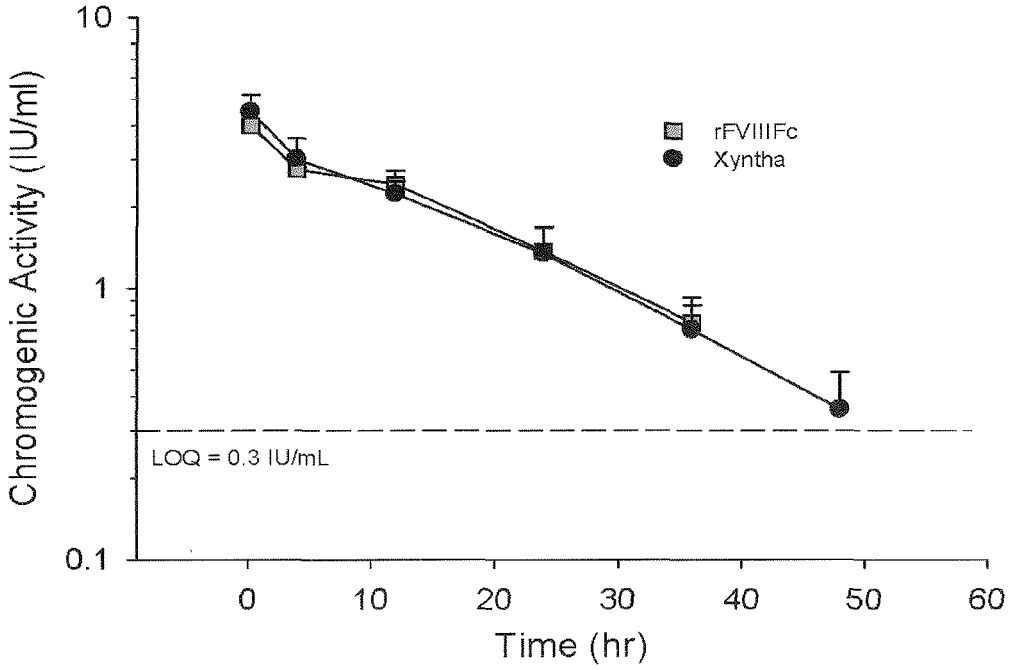
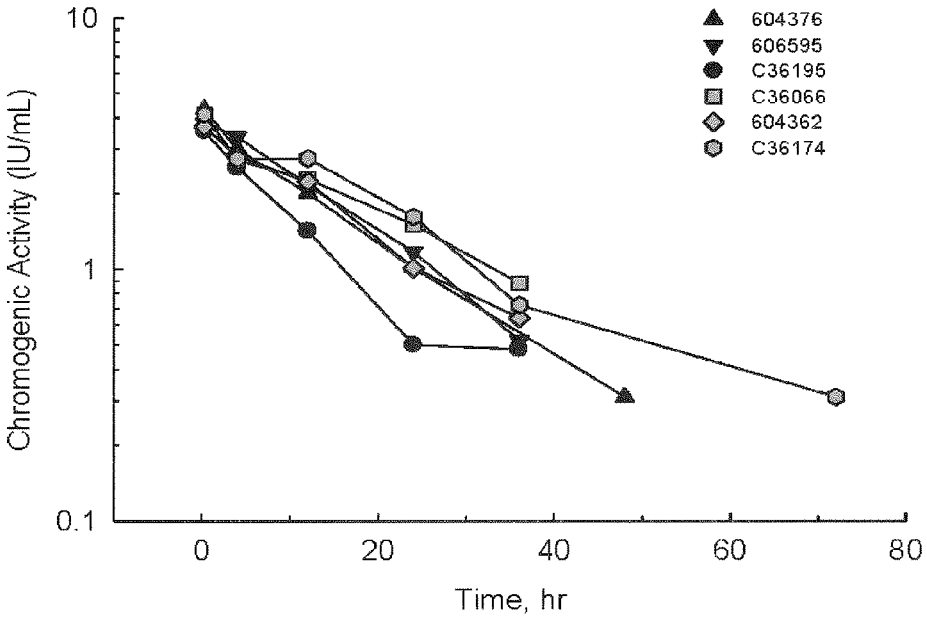

10
1
0.1
Chromogenic Activity (IU/ml)
rFVIIIFc
Xyntha
LOQ = 0.3 IU/mL
0
10
20
30
40
50
60
Time (hr)

Figure 10

A.


10
1
0.1
Chromogenic Activity (IU/mL)
0
20
40
60
80
Time, hr
604376
606595
C36195
C36066
604362
C36174

B.

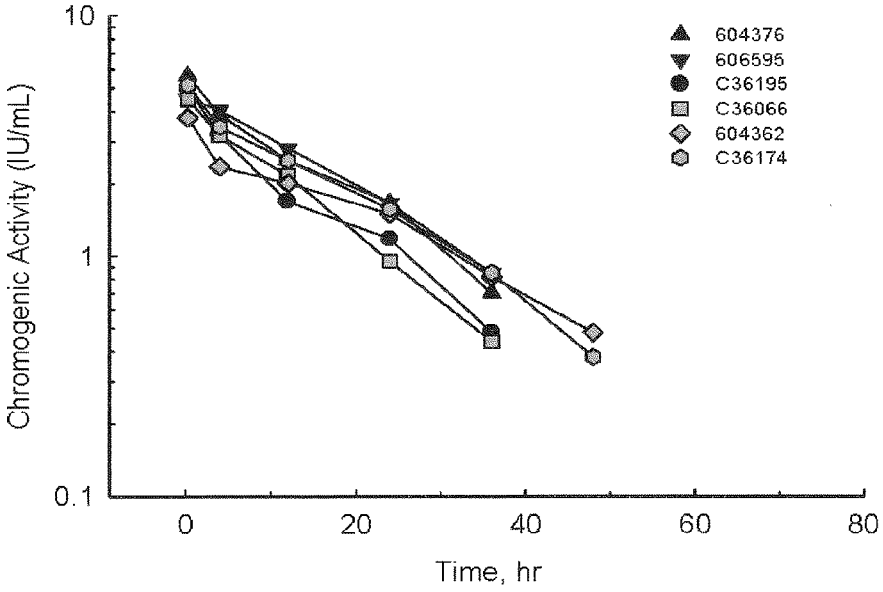

10
1
0.1
Chromogenic Activity (IU/mL)
0
20
40
60
80
Time, hr
604376
606595
C36195
C36066
604362
C36174

Figure 11

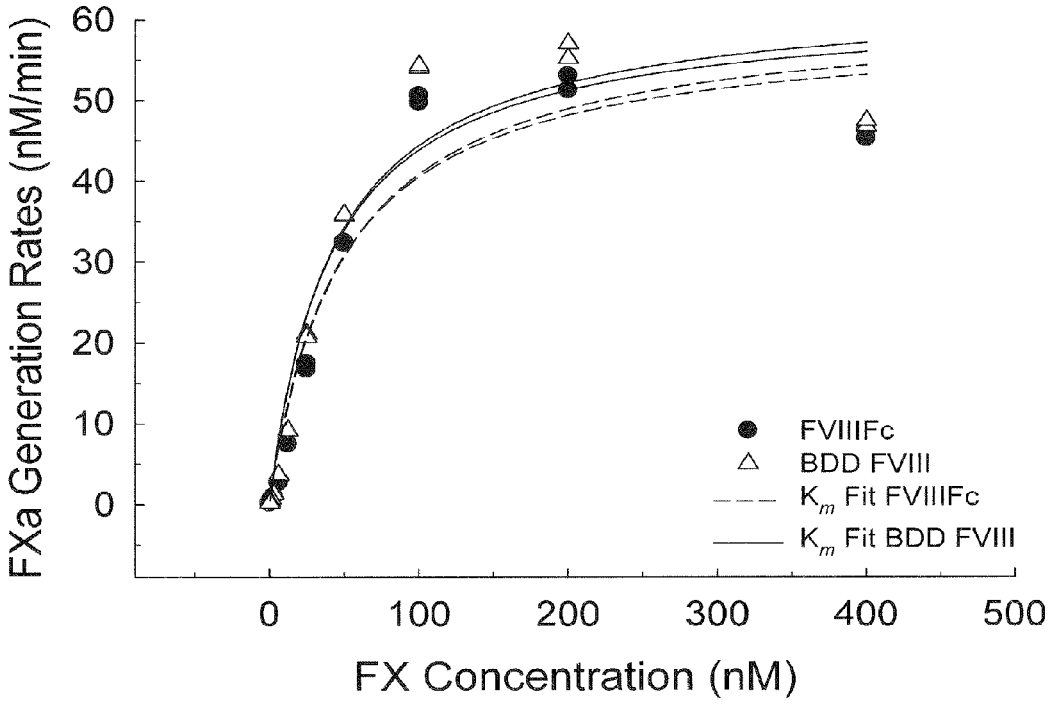

FXa Generation Rates (nM/min)
60
50
40
30
20
10
0
0
100
200
300
400
500
FX Concentration (nM)
FVIIIFc
BDD FVIII
K_m Fit FVIIIFc
K_m Fit BDD FVIII

Figure 12

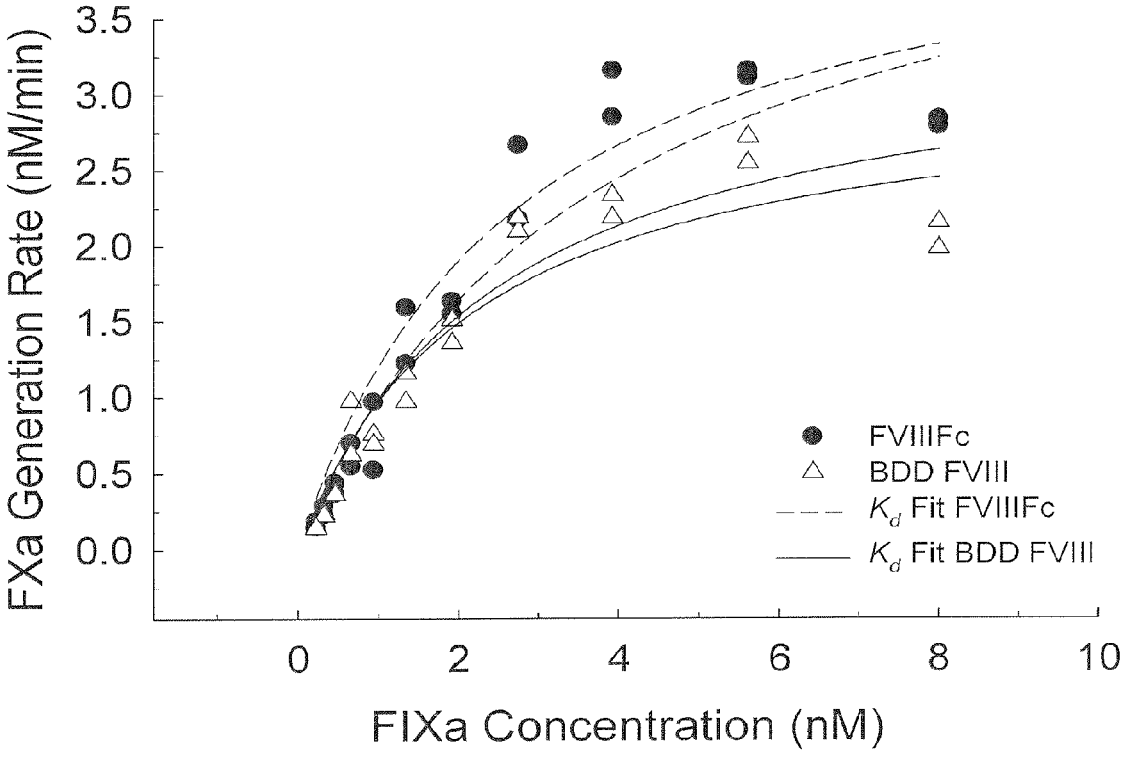
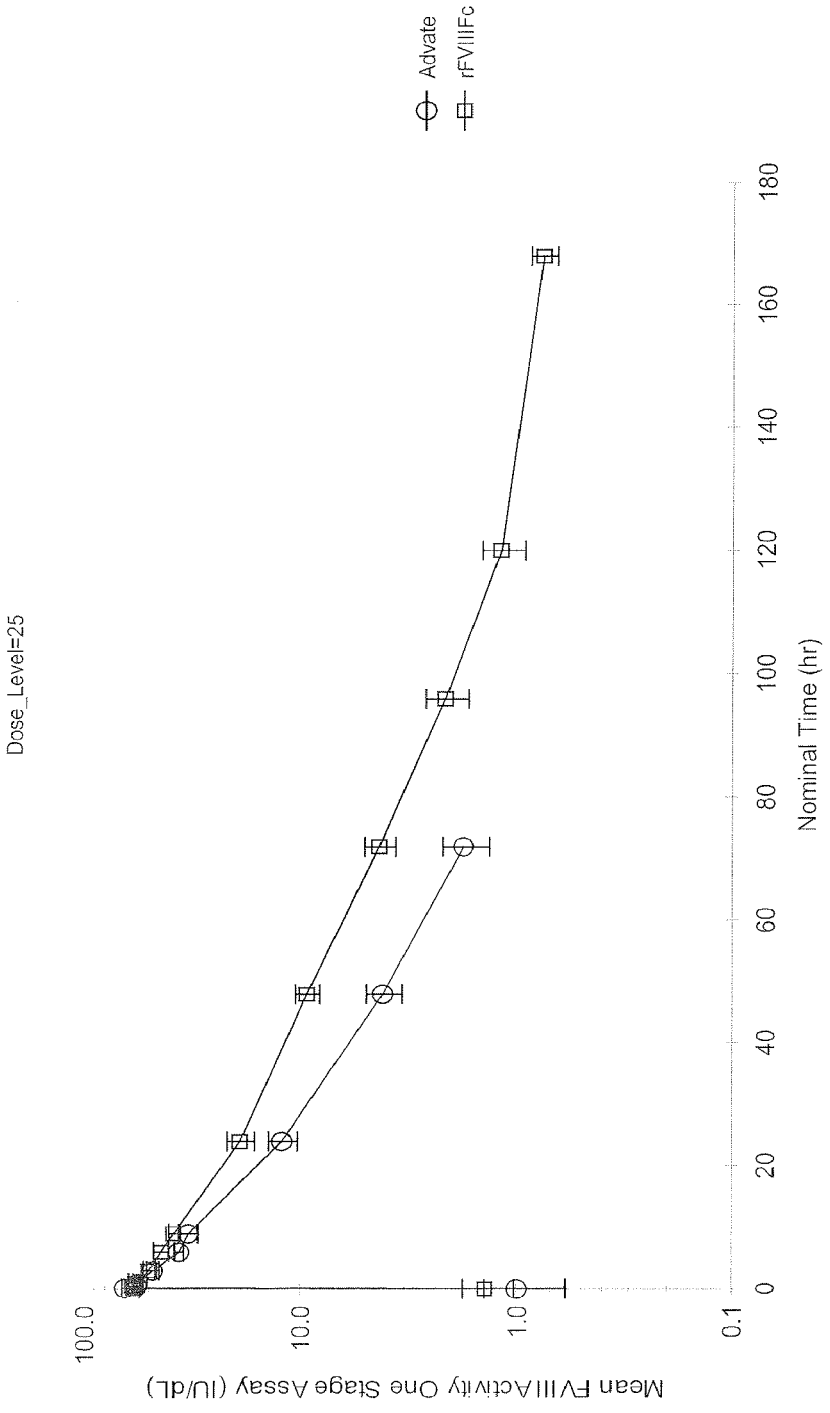

FXa Generation Rate (nM/min)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
FIXa Concentration (nM)
FVIIIFc
BDD FVIII
K_d Fit FVIIIFc
K_d Fit BDD FVIII

Figure 13A


Dose_Level=25
Advate
rFVIIIFc
Mean FVIII Activity One Stage Assay (IU/dL)
100.0
10.0
1.0
0.1
0
20
40
60
80
100
120
140
160
180
Nominal Time (hr)

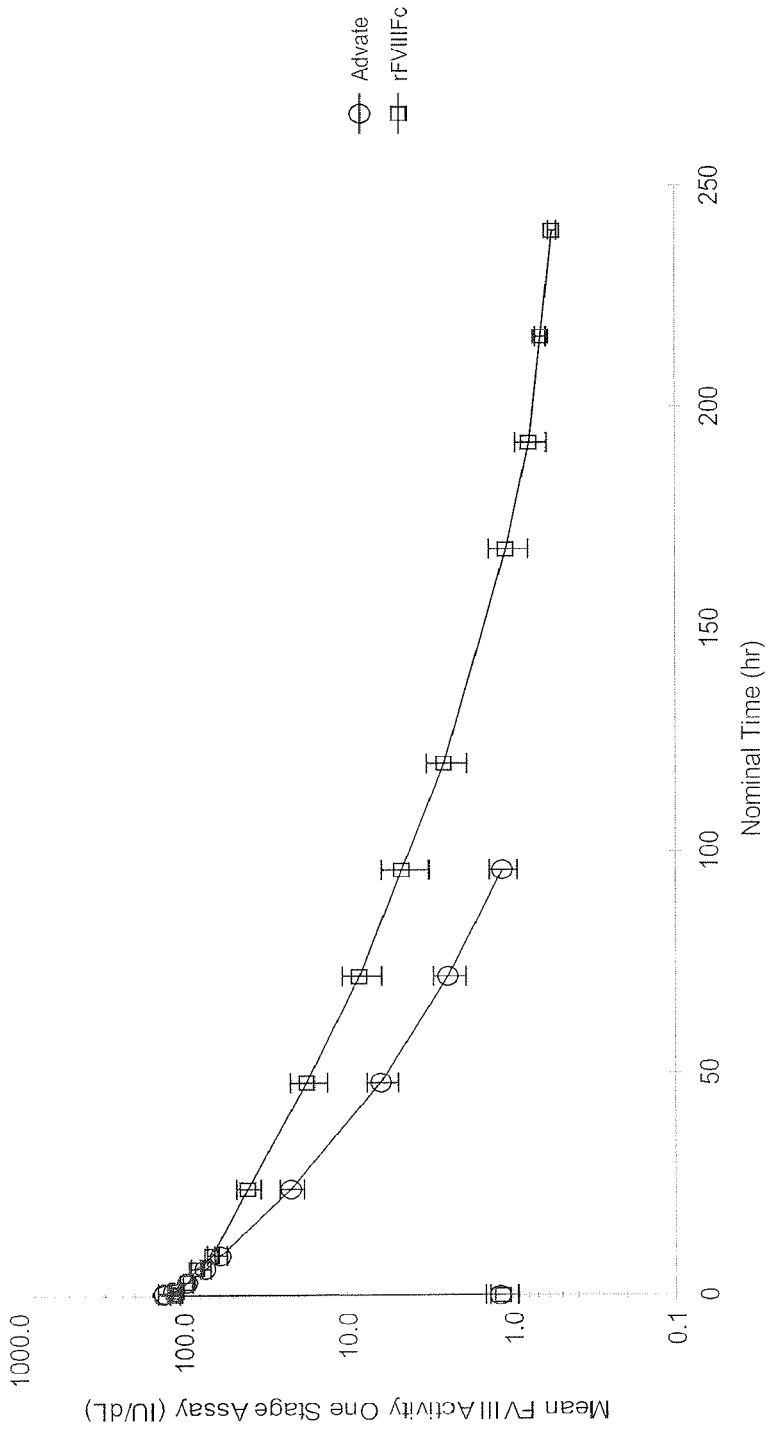
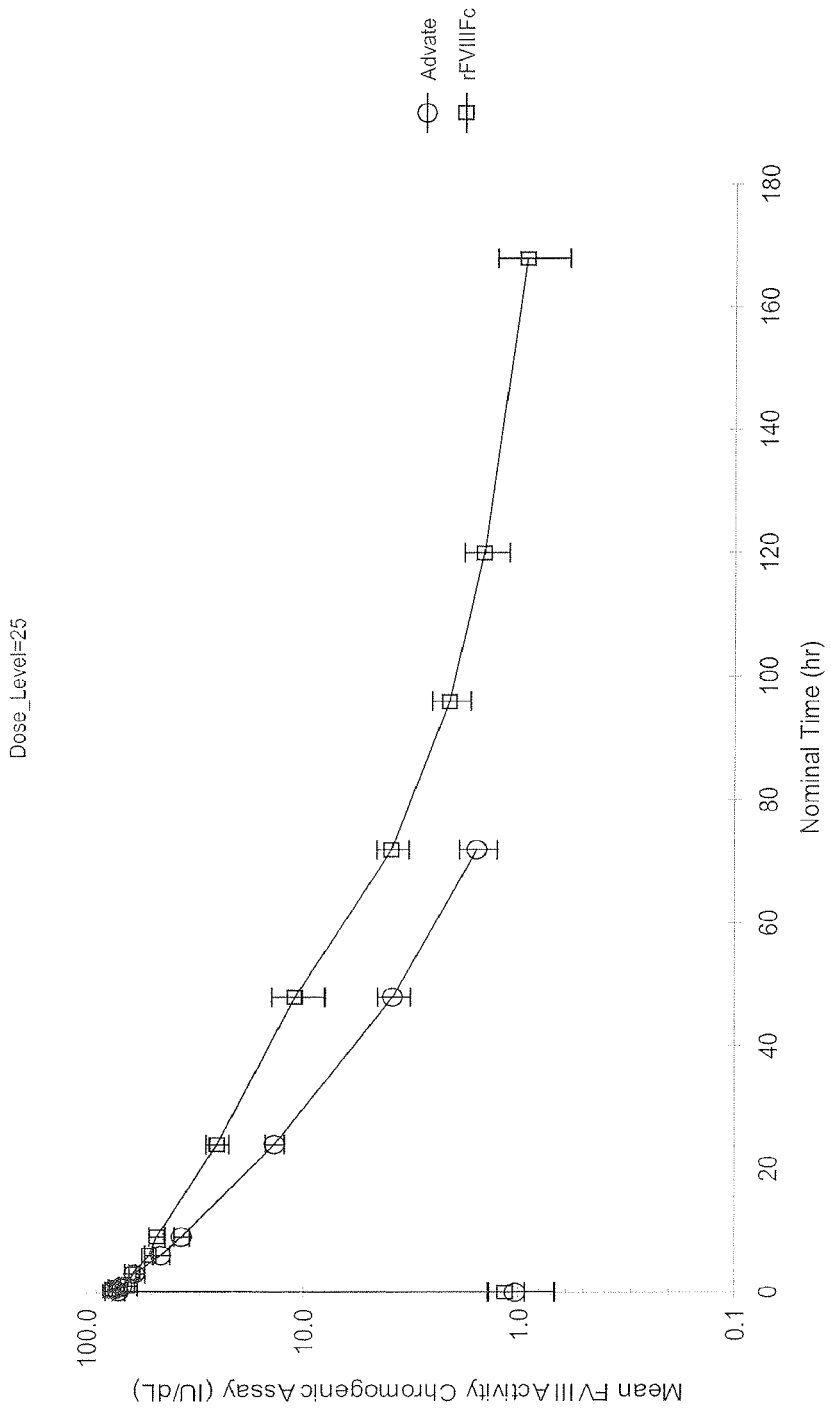


Figure 13B

Dose_Level=65

Figure 13C


Dose_Level=25
Advate
rFVIIIFc
Mean FVIII Activity Chromogenic Assay (IU/dL)
100.0
10.0
1.0
0.1
0
20
40
60
80
100
120
140
160
180
Nominal Time (hr)

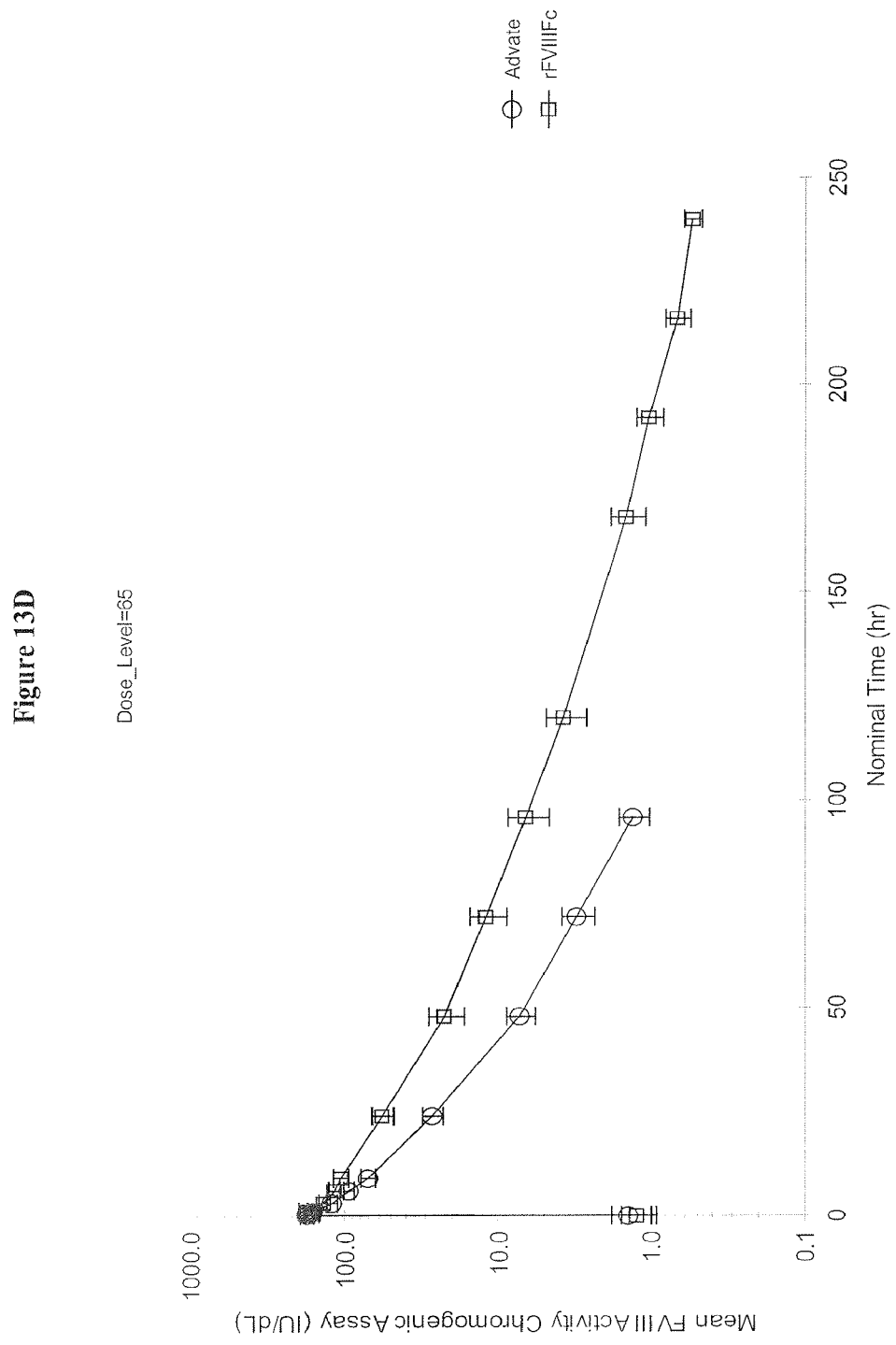

Advate
rFVIIIFc
Dose_Level=65
Mean FVIII Activity Chromogenic Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)

Figure 13D

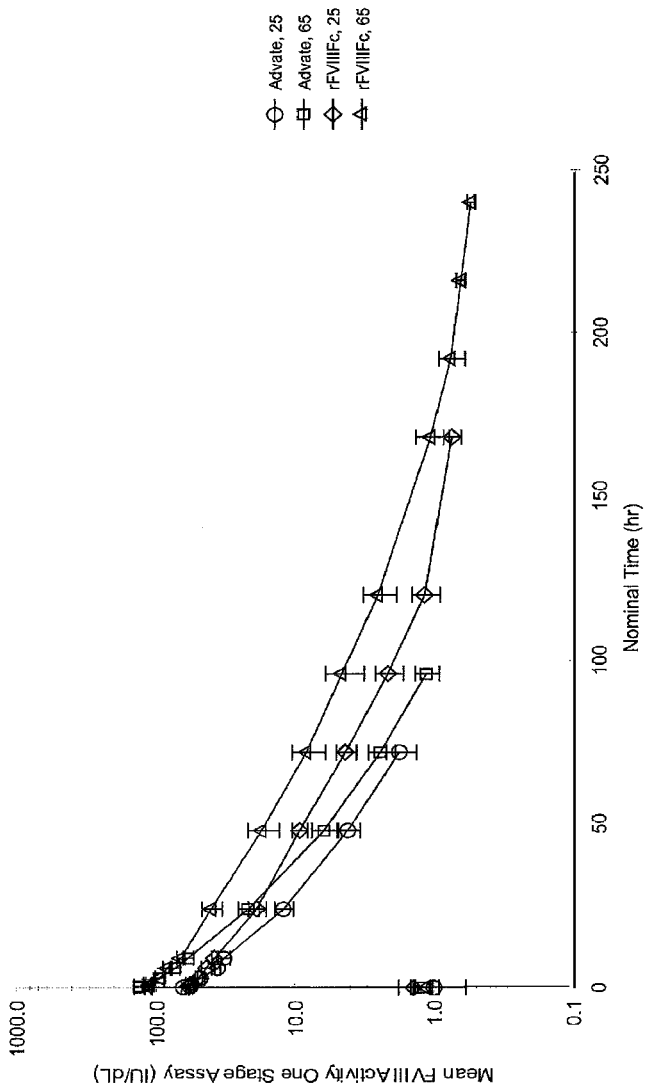
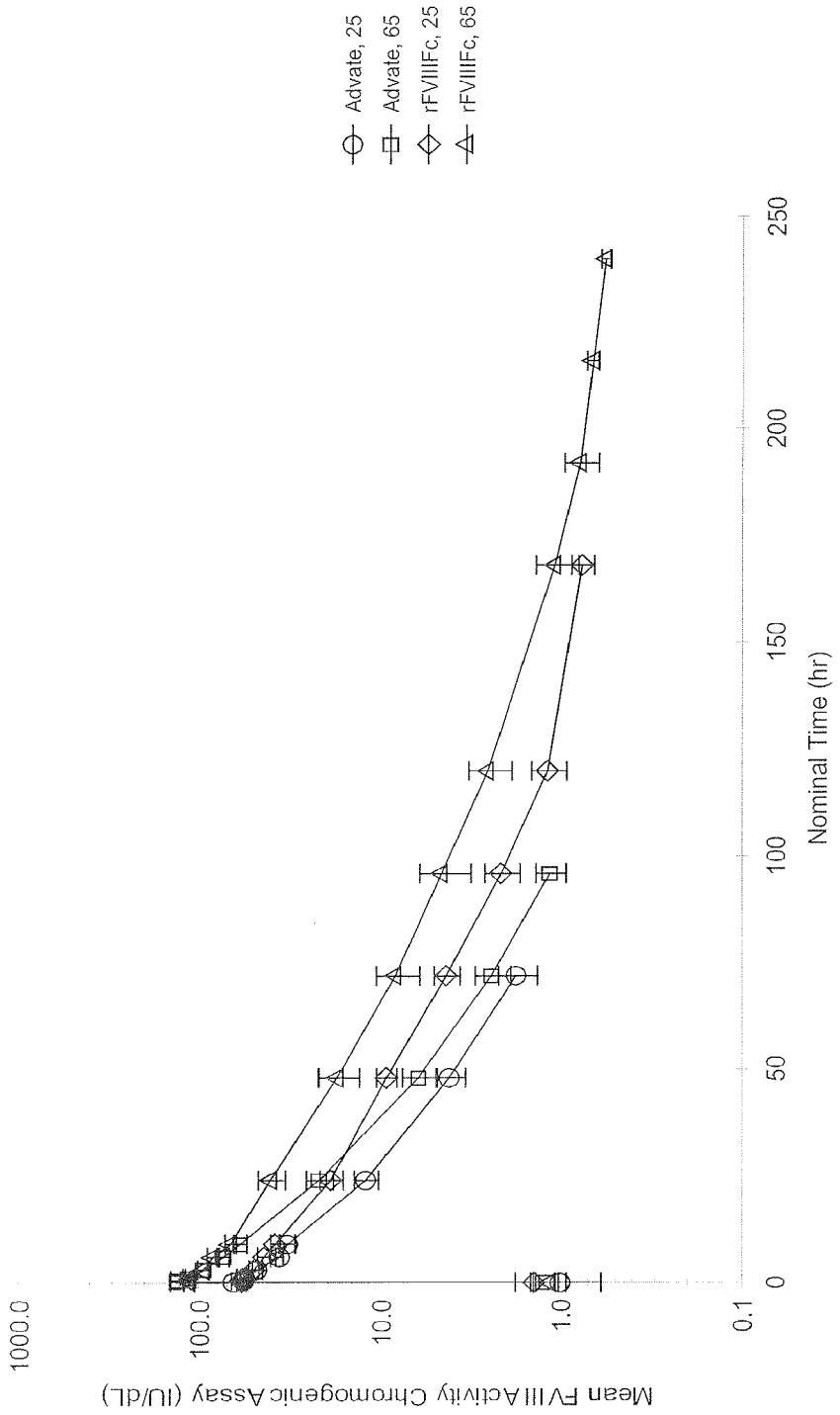

Advate, 25
Advate, 65
rFVIIIFc, 25
rFVIIIFc, 65
Mean FVIII Activity One Stage Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)

Figure 14A

Figure 14B


Advate, 25
Advate, 65
rFVIIIFc, 25
rFVIIIFc, 65
Mean FVIII Activity Chromogenic Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)

FACTOR VIII-FC CHIMERIC AND HYBRID POLYPEPTIDES, AND METHODS OF USE THEREOF

REFERENCE TO EARLIER FILED APPLICATIONS

This application is the national phase application of International Application No. PCT/US2010/059136, filed Dec. 6, 2010 and published as WO 2011/069164, which claims the benefit of U.S. Provisional Application No. 61/267,070, filed Dec. 6, 2009, U.S. Provisional Application No. 61/285,054, filed Dec. 9, 2009, U.S. Provisional Application No. 61/301,592, filed Feb. 4, 2010, U.S. Provisional Application No. 61/363,065, filed Jul. 9, 2010, U.S. Provisional Application No. 61/373,113, filed Aug. 12, 2010, U.S. Provisional Application No. 61/410,929, filed Nov. 7, 2010, and U.S. Provisional Application No. 61/419,676, filed Dec. 3, 2010, all of which are incorporated herein by reference in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

The content of the electronically submitted sequence listing (Name: 2159 2740007 SequenceListing ST25.txt, Size: 97,599 bytes; and Date of Creation: Jan. 15, 2015) submitted in this application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of therapeutics for hemostatic disorders.

2. Background Art

Hemophilia A is an X-linked bleeding disorder caused by mutations and/or deletions in the factor VIII (FVIII) gene resulting in a deficiency of FVIII activity (Peyvandi et al. 2006). The disease is characterized by spontaneous hemorrhage and excessive bleeding after trauma. Over time, the repeated bleeding into muscles and joints, which often begins in early childhood, results in hemophilic arthropathy and irreversible joint damage. This damage is progressive and can lead to severely limited mobility of joints, muscle atrophy and chronic pain (Rodriguez-Merchan, E. C., Semin. Thromb. Hemost. 29:87-96 (2003), which is herein incorporated by reference in its entirety).

The A2 domain is necessary for the procoagulant activity of the factor VIII molecule. Studies show that porcine factor VIII has six-fold greater procoagulant activity than human factor VIII (Lollar, P., and E. T. Parker, J. Biol. Chem. 266: 12481-12486 (1991)), and that the difference in coagulant activity between human and porcine factor VIII appears to be based on a difference in amino acid sequence between one or more residues in the human and porcine A2 domains (Lollar, P., et al., J. Biol. Chem. 267:23652-23657 (1992)), incorporated herein by reference in its entirety.

Treatment of hemophilia A is by replacement therapy targeting restoration of FVIII activity to 1 to 5% of normal levels to prevent spontaneous bleeding (Mannucci, P. M., et al., N. Engl. J. Med. 344:1773-1779 (2001), which is herein incorporated by reference in its entirety). There are plasma-derived and recombinant FVIII products available to treat bleeding episodes on-demand or to prevent bleeding episodes from occurring by treating prophylactically. Based on the half-life of these products treatment regimens require frequent intravenous administration. Such frequent administration is painful and inconvenient.

Reduced mortality, prevention of joint damage and improved quality of life have been important achievements due to the development of plasma-derived and recombinant FVIII. Prolonged protection from bleeding would represent another key advancement in the treatment of hemophilia A patients. However, to date, no products that allow for prolonged protection have been developed. Therefore, there remains a need for improved methods of treating hemophilia due to factor VIII deficiency that are more tolerable and more effective than current therapies.

BRIEF SUMMARY OF THE INVENTION

The present invention provides methods of administering Factor VIII; methods of administering chimeric polypeptides comprising Factor VIII and hybrids of such chimeric polypeptides; chimeric polypeptides comprising Factor VIII and hybrids of such chimeric polypeptides; polynucleotides encoding such chimeric and hybrid polypeptides; cells comprising such polynucleotides; and methods of producing such chimeric and hybrid polypeptides using such cells.

The present invention provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, at a dosing interval at least about one and one-half times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., the Fc portion. The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., the Fc portion. The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer.

The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, to obtain an area under the plasma concentration versus time curve (AUC) at least about one and one-quarter times greater than the AUC obtained by an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a polypeptide comprising a Factor VIII and an Fc at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

The methods of the invention may be practiced on a subject in need of prophylactic treatment or on-demand treatment.

On-demand treatment includes treatment for a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis (head trauma), gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the illiopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, e.g., minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

For on-demand treatment, the dosing interval of said chimeric polypeptide is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

The therapeutic doses that may be used in the methods of the invention are about 10 to about 100 IU/kg, more specifically, about 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg, and more specifically, about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

The therapeutic doses that may be used in the methods of the invention are about 10 to about 150 IU/kg, more specifically, about 100-110, 110-120, 120-130, 130-140, 140-150 IU/kg, and more specifically, about 110, 115, 120, 125, 130, 135, 140, 145, or 150 IU/kg.

The subject in the methods of the invention may be a human subject or may be a non-human mammal. Non-human mammals include, e.g., mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals. The determination of dosing interval and AUC may be carried out in a single subject or in a population of subjects.

The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be a human Factor VIII, or a non-human Factor VIII, such as porcine, mouse or canine factor VIII. The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may have a full or partial deletion of the B domain.

The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids −19 to 1438 of SEQ ID NO:2; amino acids −19 to 2332 of SEQ ID NO:6; amino acids −19 to 740 of SEQ ID NO:8; amino acids −19 to 745 of SEQ ID NO:10; or amino acids −20 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids −19 to 1438 of SEQ ID NO:2; amino acids −19 to 2332 of SEQ ID NO:6; amino acids −19 to 740 of SEQ ID NO:8; amino acids −19 to 745 of SEQ ID NO:10; or amino acids −20 to 684 of SEQ ID NO:12).

The Fc portion (or Fc portion of a chimeric polypeptide) may be at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12). The Fc portion (or Fc portion of a chimeric polypeptide) may be identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

The chimeric polypeptide may comprise a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids −19 to 1665 of SEQ ID NO:2). The chimeric polypeptide may comprise a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids −19 to 1665 of SEQ ID NO:2).

The chimeric polypeptide may be in the form of a hybrid comprising a second polypeptide in association with said chimeric polypeptide, wherein said second polypeptide comprises or consists essentially of an Fc.

The second polypeptide may comprise or consist essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids −20 to 227 of SEQ ID NO:4). The second polypeptide may comprise or consist essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids −20 to 227 of SEQ ID NO:4).

The chimeric polypeptide or hybrid may be administered as part of a pharmaceutical composition comprising at least one excipient.

The invention also provides the above-described chimeric and hybrid polypeptides themselves, polynucleotides encoding them, a cultured human embryonic cells comprising the polynucleotides, and methods of producing such chimeric and hybrid polypeptides, and the polypeptides produced by such methods.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

FIG. **1**. Schematic Representation of rFVIIIFc monomer.

FIG. **2**. WBCT of rFVIIIFc compared to ReFacto® in hemophilia A mice after a 50 IU/kg intravenous dose (n=6 mice per group).

FIG. **3**. Chromogenic Activity in Plasma from hemophilia A mice after a single IV dose of 50 IU/kg rFVIIIFc, ReFacto® and Advate®.

FIG. **4**. WBCT of rFVIIIFc and ReFacto® in hemophilia A dogs (A) rFVIIIFc. (B) ReFacto® followed by rFVIIIFc in a Crossover Study.

FIG. **5**. Pharmacokinetics of intravenous rFVIIIIFc and ReFacto® in Hemophilia A Dogs (measured by ELISA).

FIG. **6**. Activity of rFVIII and ReFacto® after a single intravenous dose in hemophilia A dogs (measured by FVIII-specific chromogenic activity assay).

FIG. **7**. Group mean plasma concentration over time of rFVIIIFc and Xyntha after a single intravenous dose (125 IU/kg) in cynomolgus monkeys (n=6, mean±SD). Plasma concentrations were measured by ELISA.

FIG. **8**. Individual plasma concentration versus time curves of rFVIIIFc and Xyntha after a single intravenous dose (125 IU/kg) in cynomolgus monkeys (n=6, mean±SD). Plasma concentrations were measured by ELISA. (A) rFVIIIFc by ELISA. (B) Xyntha by ELISA.

FIG. **9**. Group mean plasma chromogenic activity after a single intravenous dose (125 IU/kg) of rFVIIIFc and Xyntha in cynomolgus monkeys (n=6, mean±SD). FVIII activity was measured using a FVIII-specific chromogenic activity assay.

FIG. **10**. Individual plasma chromogenic activity versus time curves after a single intravenous dose (125 IU/kg) of rFVIIIFc and Xyntha in cynomolgus monkeys (n=6, mean±SD). FVIII activity was measured using a FVIII-specific chromogenic activity assay. (A) rFVIIIFc Chromogenic Activity. (B) Xyntha Chromogenic Activity.

FIG. **11**. Biochemical characterization of rFVIII-Fc: Activation of Factor X as a function of Factor X concentration.

FIG. **12**. Biochemical characterization of rFVIII-Fc: Activation of Factor X as a function of Factor IXa concentration.

FIG. **13**. Observed group mean FVIII activity (±SE) (one stage assay, 25 IU/kg (A) or 65 IU/kg (B); and chromogenic assay, 25 IU/kg (C) or 65 IU/kg (D)) versus time.

FIG. **14**. Observed group mean FVIII activity (±SE) (one stage assay (A) or chromogenic assay (B)) versus time.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of treating Hemophilia A with Factor VIII using a longer dosing interval and/or greater AUC than is possible with currently known Factor VIII products. The present invention also provides improved Factor VIII chimeric polypeptides, Factor VIII chimeric polynucleotides, and methods of production.

Treatment of hemophilia A is by replacement therapy targeting restoration of FVIII activity to 1 to 5% of normal levels to prevent spontaneous bleeding (Mannucci, P. M., et al., N. Engl. J. Med. 344:1773-9 (2001), herein incorporated by reference in its entirety). There are plasma-derived and recombinant FVIII products available to treat bleeding episodes on-demand or to prevent bleeding episodes from occurring by treating prophylactically. Based on the half-life of these products (10-12 hr) (White G. C., et al., Thromb. Haemost. 77:660-7 (1997); Morfini, M., Haemophilia 9 (suppl 1):94-99; discussion 100 (2003)), treatment regimens require frequent intravenous administration, commonly two to three times weekly for prophylaxis and one to three times daily for on-demand treatment (Manco-Johnson, M. J., et al., N. Engl. J. Med. 357:535-544 (2007)), each of which is incorporated herein by reference in its entirety. Such frequent administration is painful and inconvenient.

The present invention provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, or a hybrid of such a polypeptide at a dosing interval at least about one and one-half times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion. The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion. The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer.

The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, or a hybrid of such a polypeptide to obtain an area under the plasma concentration versus time curve (AUC) at least about one and one-quarter times greater than the AUC obtained by an equivalent amount of said Factor VIII without non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a polypeptide comprising a Factor VIII and an Fc or a hybrid of such a polypeptide at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

The methods of the invention may be practiced on a subject in need of prophylactic treatment or on-demand treatment.

"Administering," as used herein, means to give a pharmaceutically acceptable Factor VIII polypeptide of the invention to a subject via a pharmaceutically acceptable route. Preferred routes of administration are intravenous, e.g., intravenous injection and intravenous infusion. Additional routes of administration include, e.g., subcutaneous, intramuscular, oral, nasal, and pulmonary administration. Chimeric polypeptides and hybrid proteins may be administered as part of a pharmaceutical composition comprising at least one excipient.

"Area under the plasma concentration versus time curve (AUC)," as used herein, is the same as the term of art in pharmacology, and is based upon the rate and extent of absorption if factor VIII following administration. AUC is determined over a specified time period, such as 12, 18, 24, 36, 48, or 72 hours, or for infinity using extrapolation based on the slope of the curve. Unless otherwise specified herein, AUC is determined for infinity. The determination of AUC may be carried out in a single subject, or in a population of subjects for which the average is calculated.

"B domain" of Factor VIII, as used herein, is the same as the B domain known in the art that is defined by internal amino acid sequence identity and sites of proteolytic cleavage by thrombin, e.g., residues Ser741-Arg1648 of full length human factor VIII. The other human factor VIII domains are defined by the following amino acid residues: A1, residues

Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Ile2032; C1, residues Arg2033-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the factor VIII light chain activation peptide. The locations of the boundaries for all of the domains, including the B domains, for porcine, mouse and canine factor VIII are also known in the art. Preferably, the B domain of Factor VIII is deleted ("B domain deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO (recombinant BDD FVIII), which has the same sequence as the Factor VIII portion of the sequence in Table 2A(i) (amino acids −19 to 1438 or 1 to 1438 of SEQ ID NO:2).

A "B domain deleted factor VIII" may have the full or partial deletions disclosed in U.S. Pat. Nos. 6,316,226, 6,346,513, 7,041,635, 5,789,203, 6,060,447, 5,595,886, 6,228,620, 5,972,885, 6,048,720, 5,543,502, 5,610,278, 5,171,844, 5,112,950, 4,868,112, and 6,458,563, each of which is incorporated herein by reference in its entirety. In some embodiments, a B domain deleted factor VIII sequence of the present invention comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and examples 1-5 of U.S. Pat. No. 6,316,226 (also in U.S. Pat. No. 6,346,513). In some embodiments, a B domain deleted factor VIII of the present invention has a deletion disclosed at col. 2, lines 26-51 and examples 5-8 of U.S. Pat. No. 5,789,203 (also U.S. Pat. No. 6,060,447, U.S. Pat. No. 5,595,886, and U.S. Pat. No. 6,228,620). In some embodiments, a B domain deleted factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of U.S. Pat. No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Pat. No. 6,048,720; col. 2, lines 17-46 of U.S. Pat. No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Pat. No. 5,171,844; col. 2, lines 55-68, FIG. 2, and example 1 of U.S. Pat. No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Pat. No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Pat. No. 7,041,635; or col. 4, lines 25-53, of U.S. Pat. No. 6,458,563. In some embodiments, a B domain deleted factor VIII has a deletion of most of the B domain, but still contains amino-terminal sequences of the B domain that are essential for in vivo proteolytic processing of the primary translation product into two polypeptide chain, as disclosed in WO 91/09122, which is incorporated herein by reference in its entirety. In some embodiments, a B domain deleted factor VIII is constructed with a deletion of amino acids 747-1638, i.e., virtually a complete deletion of the B domain. Hoeben R. C., et al, *J. Biol. Chem.* 265 (13): 7318-7323 (1990), incorporated herein by reference in its entirety. A. B domain deleted factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of factor VIII. Meulien P., et al. *Protein Eng.* 2(4): 301-6 (1988), incorporated herein by reference in its entirety. Additional B domain deletions that are part of the invention include, e.g.: deletion of amino acids 982 through 1562 or 760 through 1639 (Toole et al., Proc. Natl. Acad. Sci. U.S.A. (1986) 83, 5939-5942)), 797 through 1562 (Eaton, et al. Biochemistry (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, et al., DNA (1987) 6:553-564)), 741 though 1648 (Pasek (PCT application No. 88/00831)), 816 through 1598 or 741 through 1689 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)), each of which is incorporated herein by reference in its entirety. Each of the foregoing deletions may be made in any Factor VIII sequence.

"Chimeric polypeptide," as used herein, means a polypeptide that includes within it at least two polypeptides (or subsequences or peptides) from different sources. Chimeric polypeptides may include, e.g., two, three, four, five, six, seven, or more polypeptides from different sources, such as different genes, different cDNAs, or different animal or other species. Chimeric polypeptides may include, e.g., one or more linkers joining the different subsequences. Thus, the subsequences may be joined directly or they may be joined indirectly, via linkers, or both, within a single chimeric polypeptide. Chimeric polypeptides may include, e.g., additional peptides such as signal sequences and sequences such as 6His and FLAG that aid in protein purification or detection. In addition, chimeric polypeptides may have amino acid or peptide additions to the N- and/or C-termini.

In some embodiments, the chimeric polypeptide comprises a Factor VIII portion and a non-Factor VIII portion. Exemplary non-Factor VIII portions include, e.g., Fc, XTEN, and albumin. Exemplary chimeric polypeptides of the invention include, e.g., chimeric Factor VIII-Fc polypeptides, chimeric Factor VIII-XTEN polypeptides, and chimeric Factor VIII-albumin polypeptides.

Exemplary chimeric Factor VIII-Fc polypeptides include, e.g., SEQ ID NOs:2, 6, 8, 10, and 12 (Table 2), with or without their signal sequences and the chimeric Fc polypeptide of SEQ ID NO:4 (Table 2).

The chimeric polypeptide may comprise a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids −19 to 1665 of SEQ ID NO:2). The chimeric polypeptide may comprise a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids −19 to 1665 of SEQ ID NO:2).

As discussed above, exemplary chimeric polypeptides include Factor VIII fused to one or more XTEN polypeptides. Schellenburger et al., Nat. Biotech. 27:1186-90 (2009), which is incorporated herein by reference in its entirety. Factor VIII can be fused to either the N-terminal end of the XTEN polypeptide or to the C-terminal end of the XTEN polypeptide, provided the Factor VIII component of the Factor VIII-XTEN fusion protein can be processed by an protease to yield a processed Factor VIII containing polypeptide. A protease site may be included between the XTEN portion and the Factor VIII portion to allow such processing. XTEN polypeptides include, e.g., those disclosed in WO 2009/023270, WO 2010/091122, WO 2007/103515, US 2010/0189682, and US 2009/0092582, each of which is incorporated herein by reference in its entirety.

As discussed above, exemplary chimeric polypeptides also include Factor VIII fused to one or more albumin polypeptides. Preferably the albumin is human albumin. Factor VIII can be fused to either the N-terminal end of the albumin or to the C-terminal end of the albumin, provided the Factor VIII component of the Factor VIII-albumin fusion protein can be processed by an enzymatically-active proprotein convertase to yield a processed Factor VIII-containing polypeptide. Examples of albumin, e.g., fragments thereof, that may be used in the present invention are known. e.g., U.S. Pat. No. 7,592,010; U.S. Pat. No. 6,686,179; and Schulte, Thrombosis Res. 124 Suppl. 2:S6-S8 (2009), each of which is incorporated herein by reference in its entirety.

In some embodiments, a chimeric polypeptide comprising a Factor VIII portion has an increased half-life (t½) over a

polypeptide consisting of the same Factor VIII portion without the non Factor VIII portion. A chimeric Factor VIII polypeptide with an increased t½ may be referred to herein as a long-acting Factor VIII. Long-acting chimeric Factor VIII polypeptides include, e.g., Factor VIII fused to Fc (including, e.g., chimeric Factor VIII polypeptides in the form of a hybrid such as a FVIIIFc monomer dimer hybrid; see Example 1, FIG. **1**, and Table 2A; and U.S. Pat. Nos. 7,404,956 and 7,348,004), Factor VIII fused to XTEN, and Factor VIII fused to albumin.

"Culture," "to culture" and "culturing," as used herein, means to incubate cells under in vitro conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated in vitro.

"Factor VIII," as used herein, means functional factor VIII polypeptide in its normal role in coagulation, unless otherwise specified. Thus, the term Factor VIII includes variant polypeptides that are functional. Preferred factor VIII proteins are the human, porcine, canine, and murine factor VIII proteins. As described in the Background Art section, the full length polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Examples of human factor VIII sequences are shown as subsequences in SEQ ID NOs:2, 6, 8, 10, and 12 (Table 2). Factor VIII polypeptides include, e.g., full-length factor VIII, full-length factor VIII minus Met at the N-terminus, mature factor VIII (minus the signal sequence), mature factor VIII with an additional Met at the N-terminus, and/or factor VIII with a full or partial deletion of the B domain. Preferred Factor VIII variants include B domain deletions, whether partial or full deletions.

A great many functional factor VIII variants are known, as is discussed above and below. In addition, hundreds of non-functional mutations in factor VIII have been identified in hemophilia patients, and it has been determined that the effect of these mutations on factor VIII function is due more to where they lie within the 3-dimensional structure of factor VIII than on the nature of the substitution (Cutler et al., Hum. Mutat. 19:274-8 (2002)), incorporated herein by reference in its entirety. In addition, comparisons between factor VIII from humans and other species has identified conserved residues that are likely to be required for function (Cameron et al., Thromb. Haemost. 79:317-22 (1998); U.S. Pat. No. 6,251,632), incorporated herein by reference in its entirety.

The human factor VIII gene was isolated and expressed in mammalian cells (Toole, J. J., et al., Nature 312:342-347 (1984); Gitschier, J., et al., Nature 312:326-330 (1984); Wood, W. I., et al., Nature 312:330-337 (1984); Vehar, G. A., et al., Nature 312:337-342 (1984); WO 87/04187; WO 88/08035; WO 88/03558; U.S. Pat. No. 4,757,006), each of which is incorporated herein by reference in its entirety, and the amino acid sequence was deduced from cDNA. Capon et al., U.S. Pat. No. 4,965,199, incorporated herein by reference in its entirety, disclose a recombinant DNA method for producing factor VIII in mammalian host cells and purification of human factor VIII. Human factor VIII expression in CHO (Chinese hamster ovary) cells and BHKC (baby hamster kidney cells) has been reported. Human factor VIII has been modified to delete part or all of the B domain (U.S. Pat. Nos. 4,994,371 and 4,868,112, each of which is incorporated herein by reference in its entirety), and replacement of the human factor VIII B domain with the human factor V B domain has been performed (U.S. Pat. No. 5,004,803, incorporated herein by reference in its entirety). The cDNA sequence encoding human factor VIII and predicted amino acid sequence are shown in SEQ ID NOs:1 and 2, respectively, of US Application Publ. No. 2005/0100990, incorporated herein by reference in its entirety.

U.S. Pat. No. 5,859,204, Lollar, J. S., incorporated herein by reference in its entirety, reports functional mutants of factor VIII having reduced antigenicity and reduced immunoreactivity. U.S. Pat. No. 6,376,463, Lollar, J. S., incorporated herein by reference in its entirety, also reports mutants of factor VIII having reduced immunoreactivity. US Application Publ. No. 2005/0100990, Saenko et al., incorporated herein by reference in its entirety, reports functional mutations in the A2 domain of factor VIII.

A number of functional factor VIII molecules, including B-domain deletions, are disclosed in the following U.S. Pat. No. 6,316,226 and U.S. Pat. No. 6,346,513, both assigned to Baxter; U.S. Pat. No. 7,041,635 assigned to In2Gen; U.S. Pat. No. 5,789,203, U.S. Pat. No. 6,060,447, U.S. Pat. No. 5,595,886, and U.S. Pat. No. 6,228,620 assigned to Chiron; U.S. Pat. No. 5,972,885 and U.S. Pat. No. 6,048,720 assigned to Biovitrum, U.S. Pat. No. 5,543,502 and U.S. Pat. No. 5,610,278 assigned to Novo Nordisk; U.S. Pat. No. 5,171,844 assigned to Immuno Ag; U.S. Pat. No. 5,112,950 assigned to Transgene S.A.; U.S. Pat. No. 4,868,112 assigned to Genetics Institute, each of which is incorporated herein by reference in its entirety.

The porcine factor VIII sequence is published, (Toole, J. J., et al., Proc. Natl. Acad. Sci. USA 83:5939-5942 (1986)), incorporated herein by reference in its entirety, and the complete porcine cDNA sequence obtained from PCR amplification of factor VIII sequences from a pig spleen cDNA library has been reported (Healey, J. F., et al., Blood 88:4209-4214 (1996), incorporated herein by reference in its entirety). Hybrid human/porcine factor VIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Pat. No. 5,364,771 by Lollar and Runge, and in WO 93/20093, incorporated herein by reference in its entirety. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine factor VIII and a chimeric factor VIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503, incorporated herein by reference in its entirety. U.S. Pat. No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. U.S. Pat. No. 6,458,563, incorporated herein by reference in its entirety assigned to Emory discloses a B-domain deleted porcine Factor VIII.

The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids −19 to 1438 of SEQ ID NO:2; amino acids −19 to 2332 of SEQ ID NO:6; amino acids −19 to 740 of SEQ ID NO:8; amino acids −19 to 745 of SEQ ID NO:10; or amino acids −20 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table

2 with a signal sequence (amino acids −19 to 1438 of SEQ ID NO:2; amino acids −19 to 2332 of SEQ ID NO:6; amino acids −19 to 740 of SEQ ID NO:8; amino acids −19 to 745 of SEQ ID NO:10; or amino acids −20 to 684 of SEQ ID NO:12).

"Equivalent amount," as used herein, means the same amount of Factor VIII activity as expressed in International Units, which is independent of molecular weight of the polypeptide in question. One International Unit (IU) of factor VIII activity corresponds approximately to the quantity of factor VIII in one milliliter of normal human plasma. Several assays are available for measuring Factor VIII activity, including the European Pharmacopoeia chromogenic substrate assay and a one stage clotting assay.

"Fc," as used herein, means functional neonatal Fc receptor (FcRn) binding partners, unless otherwise specified. An FcRn binding partner is any molecule that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the FcRn binding partner. Thus, the term Fc includes any variants of IgG Fc that are functional. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379, incorporated herein by reference in its entirety). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The FcRn binding partners include, e.g., whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of immunoglobulins or immunoglobulin fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda; MD, incorporated herein by reference in its entirety. (The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180: 2377), incorporated herein by reference in its entirety.) An Fc may comprise the CH2 and CH3 domains of an immunoglobulin with or without the hinge region of the immunoglobulin. Exemplary Fc variants are provided in WO 2004/101740 and WO 2006/074199, incorporated herein by reference in its entirety.

Fc (or Fc portion of a chimeric polypeptide) may contain one or more mutations, and combinations of mutations.

Fc (or Fc portion of a chimeric polypeptide) may contain mutations conferring increased half-life such as M252Y, S254T, T256E, and combinations thereof, as disclosed in Oganesyan et al., Mol. Immunol. 46:1750 (2009), which is incorporated herein by reference in its entirety; H433K, N434F, and combinations thereof, as disclosed in Vaccaro et al., Nat. Biotechnol. 23:1283 (2005), which is incorporated herein by reference in its entirety; the mutants disclosed at pages 1-2, paragraph [0012], and Examples 9 and 10 of US 2009/0264627 A1, which is incorporated herein by reference in its entirety; and the mutants disclosed at page 2, paragraphs [0014] to [0021] of US 20090163699 A1, which is incorporated herein by reference in its entirety.

Fc (or Fc portion of a chimeric polypeptide) may also include, e.g., the following mutations: The Fc region of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be hound by FcRn. Such modifications include, e.g., modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example the following single amino acid residues in human IgG1 Fc (Fcy1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, A330S, P331A, P331S, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wildtype proline substituted by alanine at position number 238. In addition to alanine other amino acids may be substituted for the wildtype amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred FcRn binding partners distinct from native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more FcRn binding partners. Certain of these mutations may confer new functionality upon the FcRn binding partner. For example, one embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the FcRn binding partner, and to render the FcRn binding partner incapable of binding to FcyRI, FcyRIIA, FcyRIIB, and FcyRIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847, which is incorporated herein by reference in its entirety; Friend et al. 1999, Transplantation 68:1632, which is incorporated herein by reference in its entirety; Shields et al. 1995, J. Biol. Chem. 276:6591, which is incorporated herein by reference in its entirety). Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcyRI, FcyRII, and FcyRIII which mediate various effector functions will not bind to IgG1 when such mutations have been introduced (Ward and Ghetie 1995, Therapeutic Immunology 2:77, which is incorporated herein by reference in its entirety; and Armour et al. 1999, Eur. J. Immunol. 29:2613, which is incorporated herein by reference in its entirety). As a further example of new functionality arising from mutations described above affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Mutations believed to impart an increased affinity for FcRn include, e.g., T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591, which is incorporated herein by reference in its entirety).

The Fc (or Fc portion of a chimeric polypeptide) may be at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741

to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12). The Fc (or Fc portion of a chimeric polypeptide) may be identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

"Hybrid" polypeptides and proteins, as used herein, means a combination of a chimeric polypeptide with a second polypeptide. The chimeric polypeptide and the second polypeptide in a hybrid may be associated with each other via protein-protein interactions, such as charge-charge or hydrophobic interactions. The chimeric polypeptide and the second polypeptide in a hybrid may be associated with each other via disulfide or other covalent bond(s). Hybrids are described in WO 2004/101740 and WO 2006/074199, each of which is incorporated herein by reference in its entirety. See also U.S. Pat. Nos. 7,404,956 and 7,348,004, each of which is incorporated herein by reference in its entirety. The second polypeptide may be a second copy of the same chimeric polypeptide or it may be a non-identical chimeric polypeptide. See, e.g., FIG. **1**, Example 1, and Table 2. In preferred embodiments, the second polypeptide is a polypeptide comprising an Fc. In preferred embodiments, the chimeric polypeptide is a chimeric Factor VIII-Fc polypeptide and the second polypeptide consists essentially of Fc, e.g, the hybrid polypeptide of Example 1, which is a rFVIIIFc recombinant fusion protein consisting of a single molecule of recombinant B-domain deleted human FVIII (BDD-rFVIII) fused to the dimeric Fc domain of the human IgG1, with no intervening linker sequence. This hybrid polypeptide is referred to herein as FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc monomer-dimer. See Example 1, FIG. **1**, and Table 2A. The Examples provide preclinical and clinical data for this hybrid polypeptide.

The second polypeptide in a hybrid may comprise or consist essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids −20 to 227 of SEQ ID NO:4). The second polypeptide may comprise or consist essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids −20 to 227 of SEQ ID NO:4).

FIG. **1** is a schematic showing the structure of a B domain deleted factor VIII-Fc chimeric polypeptide, and its association with a second polypeptide that is an Fc polypeptide. To obtain this hybrid, the coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A RNA (Clontech) using FVIII-specific primers. The FVIII sequence includes the native signal sequence for FVIII. The B-domain deletion was from serine 743 (S743; 2287 bp) to glutamine 1638 (Q1638; 4969 bp) for a total deletion of 2682 bp. Then, the coding sequence for human recombinant Fc was obtained by RT-PCR from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were designed such that the B-domain deleted FVIII sequence was fused directly to the N-terminus of the Fc sequence with no intervening linker. The FVIIIFc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter. A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1α, in the expression vector pBUDCE4.1.

The rFVIIIFc expression vector was transfected into human embryonic kidney 293 cells (HEK293H; Invitrogen) using Lipofectamine 2000 transfection reagent (Invitrogen). Stable clonal cell lines were generated by selection with Zeocin (Invitrogen). One clonal cell line, 3C4-22 was used to generate FVIIIFc for characterization in vivo. Recombinant FVIIIFc was produced and purified (McCue et al. 2009) at Biogen Idec (Cambridge, Mass.). The transfection strategy described above was expected to yield three products, i.e., monomeric rFVIIIFc hybrids, dimeric rFVIIIFc hybrids and dimeric Fc. However, there was essentially no dimeric rFVIIIFc detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIIIFc. It is possible that the size of dimeric rFVIIIFc was too great and prevented efficient secretion from the cell. This result was beneficial since it rendered the purification of the monomer less complicated than if all three proteins had been present. The material used in these studies had a specific activity of approximately 9000 IU/mg.

"Dosing interval," as used herein, means the amount of time that elapses between multiple doses being administered to a subject. The comparison of dosing interval may be carried out in a single subject or in a population of subjects and then the average obtained in the population may be calculated.

The dosing interval when administering a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide (a polypeptide comprising a Factor VIII or a hybrid) of the invention may be at least about one and one-half times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII). The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII). The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII). The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer. The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer. For on-demand treatment, the dosing interval of said chimeric polypeptide or hybrid is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

Preferably, the effective dose is 25-65 IU/kg (25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 62, 64, or 65 IU/kg) and the dosing interval is once every 3-5, 3-6, 3-7, 3, 4, 5, 6, 7, or 8 or more days, or three times per week, or no more than three times per week. Preferably, the effective dose is 65 IU/kg and the dosing interval is once weekly, or once every 6-7 days.

"Long-acting Factor VIII" is a Factor VIII having an increased half-life (also referred to herein as t½, t½ beta, elimination half-life and HL) over a reference Factor VIII. The increased half-life of a long-acting Factor VIII may be due to fusion to one or more non-Factor VIII polypeptides such as, e.g., Fc, XTEN or albumin. The increased half-life may be due to one or more modification, such as, e.g., pegylation. Exemplary long-acting Factor VIII polypeptides include, e.g., chimeric Factor VIII polypeptides comprising Fc, chimeric Factor VIII polypeptides comprising XTEN and chimeric Factor VIII polypeptides comprising albumin. Additional exemplary long-acting Factor VIII polypeptides include, e.g., pegylated Factor VIII.

The "reference" polypeptide, in the case of a long-acting chimeric Factor VIII polypeptide, is a polypeptide consisting essentially of the Factor VIII portion of the chimeric polypeptide, e.g., the same Factor VIII portion without the Fc portion, without the XTEN portion, or without the albumin portion. Likewise, the reference polypeptide in the case of a modified Factor VIII is the same Factor VIII without the modification, e.g., a Factor VIII without the pegylation.

In some embodiments, the long-acting Factor VIII has one or more of the following properties when administered to a subject:

a mean residence time (MRT) (activity) in said subject of about 14-41.3 hours;

a clearance (CL) (activity) in said subject of about 1.22-5.19 mL/hour/kg or less;

a t½beta (activity) in said subject of about 11-26.4 hours;

an incremental recovery (K value) (activity; observed) in said subject of about 1.38-2.88 IU/dL per IU/kg;

a Vss (activity) in said subject of about 37.7-79.4 mL/kg; and

an AUC/dose in said subject of about 19.2-81.7 IU*h/dL per IU/kg.

In some embodiments, the long-acting Factor VIII has one or more of the following properties when administered to a patient population:

a mean incremental recovery (K-Value) (activity; observed) greater that 1.38 IU/dL per IU/kg;

a mean incremental recovery (K-Value) (activity; observed) of at least about 1.5, at least about 1.85, or at least about 2.46 IU/dL per IU/kg.

a mean clearance (CL) (activity) in said patient population of about 2.33±1.08 mL/hour/kg or less;

a mean clearance (CL) (activity) in said patient population of about 1.8-2.69 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population that is about 65% of the clearance of a polypeptide comprising said Factor VIII without modification;

a mean residence time (MRT) (activity) in said patient population of at least about 26.3±8.33 hours;

a mean MRT (activity) in said patient population of about 25.9-26.5 hours;

a mean MRT (activity) in said patent population that is about 1.5 fold longer than the mean MRT of a polypeptide comprising said Factor VIII without modification;

a mean t½beta (activity) in said patient population of about 18.3±5.79 hours;

a mean t½beta (activity) in said patient population that is about 18-18.4 hours;

a mean t½beta (activity) in said patient population that is about 1.5 fold longer than the mean t½beta of a polypeptide comprising said Factor VIII without modification;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 2.01±0.44 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 1.85-2.46 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population that is about 90% of the mean incremental recovery of a polypeptide comprising said Factor VIII without modification;

a mean Vss (activity) in said patient population of about 55.1±12.3 mL/kg;

a mean Vss (activity) in said patient population of about 45.3-56.1 mL/kg;

a mean AUC/dose (activity) in said patient population of about 49.9±18.2 IU*h/dL per IU/kg;

a mean AUC/dose (activity) in said patient population of about 44.8-57.6 IU*h/dL per IU/kg.

"On-demand treatment," as used herein, means treatment that is intended to take place over a short course of time and is in response to an existing condition, such as a bleeding episode, or a perceived need such as planned surgery. Conditions that may require on-demand treatment include, e.g., a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the illiopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, e.g., minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

Preferably, on-demand treatment resolves greater than 80% (greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or 100%) or 80-100%, 80-90%, 85-90%, 90-100%, 90-95%, or 95-100% of bleeds (e.g., spontaneous bleeds) in a single dose. Preferably, greater than 80% (greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or 100%) or 80-100%, 80-90%, 85-90%, 90-100%, 90-95%, or 95-100% of bleeding episodes are rated excellent or good by physicians after on-demand treatment. Preferably, greater than 5%, (greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, greater than 15%, greater than 16%, greater than 17%, greater than 18%, greater than 19%, greater than 20%), or 5-20%, 5-15%, 5-10%, 10-20%, or 10-15% of bleeding episodes are rated as fair by physicians after on-demand treatment.

"Polypeptide," "peptide" and "protein" are used interchangeably and refer to a polymeric compound comprised of covalently linked amino acid residues.

"Polynucleotide" and "nucleic acid" are used interchangeably and refer to a polymeric compound comprised of covalently linked nucleotide residues. Polynucleotides may be DNA, cDNA, RNA, single stranded, or double stranded, vectors, plasmids, phage, or viruses. Polynucleotides include,

e.g., those in Table 1, which encode the polypeptides of Table 2 (see Table 1). Polynucleotides also include, e.g., fragments of the polynucleotides of Table 1, e.g., those that encode fragments of the polypeptides of Table 2, such as the Factor VIII, Fc, signal sequence, 6His and other fragments of the polypeptides of Table 2.

"Prophylactic treatment," as used herein, means administering a Factor VIII polypeptide in multiple doses to a subject over a course of time to increase the level of Factor VIII activity in a subject's plasma. Preferably, the increased level is sufficient to decrease the incidence of spontaneous bleeding or to prevent bleeding, e.g., in the event of an unforeseen injury. Preferably, during prophylactic treatment, the plasma protein level in the subject does not fall below the baseline level for that subject, or below the level of Factor VIII that characterizes severe hemophilia (<1 IU/dl [1%]).

Preferably, the prophylaxis regimen is "tailored" to the individual patient, preferably by determining PK data for each patient and administering Factor VIII of the invention at a dosing interval that maintains a trough level of 1-3% FVIII activity. Adjustments may be made when a subject experiences unacceptable bleeding episodes defined as ≥2 spontaneous bleeding episodes over a rolling two-month period. In this case, adjustment will target trough levels of 3-5%. Preferably, prophylactic treatment results in prevention and control of bleeding, sustained control of bleeding, sustained protection from bleeding, and/or sustained benefit. Prophylaxis, e.g., sustained protection can be demonstrated by an increased AUC to last measured time point (AUC-LAST) and reduced clearance, resulting in increased terminal t½ compared to short acting FVIII. Preferably, prophylaxis is demonstrated by better Cmax, better Tmax, and/or greater mean residence time versus short-acting FVIII. Preferably, prophylaxis results in no spontaneous bleeding episodes within about 24, 36, 48, 72, or 96 hours (e.g., 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 96, 87, 88, 89, 90, 91, 92, 93, 94, 95, or 96 hours, preferably within 72 hours), after injection (e.g., the last injection). Preferably, prophylaxis results in greater than 30% (e.g., greater than 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 96, 87, 88, 89, or 90%, preferably greater than 50%), mean reduction in annualized bleeding episodes with once weekly dosing (e.g., at 65 IU/kg).

"Subject," as used herein means a human or a non-human mammal. Non-human mammals include, e.g., mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals.

"Therapeutic dose," as used herein, means a dose that achieves a therapeutic goal, as described herein. The calculation of the required dosage of factor VIII is based upon the empirical finding that, on average, 1 IU of factor VIII per kg body weight raises the plasma factor VIII activity by approximately 2 IU/dL. The required dosage is determined using the following formula:

Required units=body weight (kg)×desired factor VIII rise (IU/dL or % of normal)×0.5 (IU/kg per IU/dL)

The therapeutic doses that may be used in the methods of the invention are about 10-100 IU/kg, more specifically, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg, and more specifically, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

Additional therapeutic doses that may be used in the methods of the invention are about 10 to about 150 IU/kg, more specifically, about 100-110, 110-120, 120-130, 130-140, 140-150 IU/kg, and more specifically, about 110, 115, 120, 125, 130, 135, 140, 145, or 150 IU/kg.

"Variant," as used herein, refers to a polynucleotide or polypeptide differing from the original polynucleotide or polypeptide, but retaining essential properties thereof, e.g., factor VIII coagulant activity or Fc (FcRn binding) activity. Generally, variants are overall closely similar, and, in many regions, identical to the original polynucleotide or polypeptide. Variants include, e.g., polypeptide and polynucleotide fragments, deletions, insertions, and modified versions of original polypeptides.

Variant polynucleotides may comprise, or alternatively consist of, a nucleotide sequence which is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:1, 3, 5, 7, 9, or 11 (the factor VIII portion, the Fc portion, individually or together) or the complementary strand thereto, the nucleotide coding sequence of known mutant and recombinant factor VIII or Fc such as those disclosed in the publications and patents cited herein or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, 4, 6, 8, 10, or 12 (the factor VIII portion, the Fc portion, individually or together), and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also included as variants, as are polypeptides encoded by these polynucleotides as long as they are functional.

Variant polypeptides may comprise, or alternatively consist of, an amino acid sequence which is at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:2, 4, 6, 8, 10, or 12 (the factor VIII portion, the Fc portion, individually or together), and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be, for example, the entire sequence shown in SEQ ID NO:1 or 3, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (reference or original sequence) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990)

6:237-245), which is herein incorporated by reference in its entirety In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score-1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences of SEQ ID NO:2 (the factor VIII portion, the Fc portion, individually or together) or 4, or a known factor VIII or Fc polypeptide sequence, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (reference or original sequence) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., Comp. App. Biosci. 6:237-245 (1990), incorporated herein by reference in its entirety. In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are

not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The polynucleotide variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), incorporated herein by reference in its entirety, reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988), incorporated herein by reference in its entirety.)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993), incorporated herein by reference in its entirety) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

As stated above, polypeptide variants include, e.g., modified polypeptides. Modifications include, e.g., acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation (Mei et al., Blood 116: 270-79 (2010), which is incorporated herein by reference in its entirety), proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. In some embodiments, Factor VIII is modified, e.g., pegylated, at any convenient location. In some embodiments, Factor VIII is pegylated at a surface exposed amino acid of Factor VIII, preferably a surface exposed cysteine, which may be an engineered cysteine. Mei et al. (2010). In some embodiments, modified Factor VIII, e.g., pegylated Factor VIII, is a long-acting Factor VIII.

"Volume of distribution at steady state (Vss)," as used herein, has the same meaning as the term used in pharmacology, which is the apparent space (volume) into which a drug distributes. Vss=the amount of drug in the body divided by the plasma concentration at steady state.

"About," as used herein for a range, modifies both ends of the range. Thus, "about 10-20" means "about 10 to about 20."

Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention. All patents and publications referred to herein are expressly incorporated by reference.

Example 1

Abstract

A recombinant B-domain-deleted factor VIII-Fc (rFVIIIFc) fusion protein was created to extend the half-life of FVIII. rFVIIIFc was studied in mouse and dog models of severe hemophilia A and compared to rFVIII (ReFacto®). Whole blood clotting time (WBCT) in hemophilia A mice was corrected for approximately two to three times longer and the elimination half-life in plasma was nearly twice as long for rFVIIIFc compared to ReFacto®. In hemophilia A dogs, an intravenous dose of rFVIIIFc (125 IU/kg) corrected the WBCT to normal. The WBCT remained below 20 min, the time consistent with FVIII:C>1%, through approximately 96 hr, compared to 48 hr for dogs treated with ReFacto®. The elimination half-life of rFVIIIFc in dog plasma, when measured using ELISA or chromogenic activity assays, was 15.7±1.7 hr and 15.4±0.3 hr, respectively. ReFacto® corrected WBCT for approximately one half as long as rFVIIIFc and the plasma half-life was 7.0 hr. Thus, fusion of FVIII to Fc produced a molecule with an increased plasma half-life and the ability to provide prolonged protection from bleeding.

Introduction

Reduced mortality, prevention of joint damage and improved quality of life have been important achievements due to the development of plasma-derived and recombinant FVIII. Prolonged protection from bleeding would represent another key advancement in the treatment of hemophilia A patients. The inventors have created a recombinant factor VIII-Fc (rFVIIIFc) chimeric protein and hybrid as an approach to extend the half-life of FVIII.

rFVIIIFc is a heterodimeric hybrid protein comprised of B-domain-deleted FVIII fused recombinantly to the Fc domain of human immunoglobulin G1 (IgG1) (FIG. **1**, SEQ ID NO:2; Table 2A) (This protein is also referred to herein as FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc monomer-dimer.). The Fc enables binding to the neonatal Fc receptor (Ran), which is responsible for protection of IgG from degradation and confers on IgG the three week half-life observed in humans (Ghetie V, and Ward E S., Annu. Rev. Immunol. 2000; 18:739-766; Roopenian D C, and Akilesh S., Nature Rev. Immunol. 2007; 7:715-725, each of which is incorporated herein by reference in its entirety).

The Fc domain of IgG1 has been fused to growth factors, cytokines, enzymes and ligand-binding regions of receptors (Ashkanazi A, et al., Int. Rev. Immunol. 1993:10:219-27; Chamow S M, and Ashkanazi A, Trends Biotechnol. 1996: 14:52-60; Fisher et al., N. Engl. J. Med. 1996:334(26):1697-702, each of which is incorporated herein by reference in its entirety). Several of these have become important therapeutic molecules (e.g. etanercept, alefacept, abatacept). In these fusion proteins, two effector molecules are connected to two Fc molecules. In this example, rFVIIIFc has been constructed as a monomeric Fc fusion protein (one copy of a polypeptide consisting of the sequence in Table 2A(i) (SEQ ID NO:2) with or without the signal sequence and one copy of a polypeptide consisting of the sequence in Table 2A(ii) (SEQ ID NO:4) with or without the signal sequence), i.e., with only one copy of the effector molecule (see FIG. **1**), and the studies presented herein compare the pharmacodynamics and pharmacokinetics of this novel protein to rFVIII in mouse and dog models of hemophilia A. The signal sequence is cleavage during secretion. This protein construct is referred to herein as FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc monomer-dimer. See Example 1, FIG. **1**, Table 2A; and U.S. Pat. Nos. 7,404,956 and 7,348,004, each of which is incorporated herein by reference in its entirety, for the structure and production of this protein.

Methods and Materials

FVIII Preparations

Recombinant FVIIIFc

The coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A RNA (Clontech) using FVIII-specific primers. The FVIII sequence includes the native signal sequence for FVIII. The B-domain deletion was from serine 743 (S743; 2287 bp) to glutamine 1638 (Q1638; 4969 bp) for a total deletion of 2682 bp See Example 1, FIG. **1**, Table 2A; and U.S. Pat. Nos. 7,404,956 and 7,348,004, each of which is incorporated herein by reference in its entirety, for the structure and production of this protein.

The coding sequence for human recombinant Fc was obtained by RT-PCR from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were designed such that the B-domain deleted FVIII sequence was fused directly to the N-terminus of the Fc sequence with no intervening linker. The FVIIIFc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter. A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1α, in the expression vector pBUDCE4.1.

The rFVIIIFc expression vector was transfected into human embryonic kidney 293 cells (HEK293H; Invitrogen) using Lipofectamine 2000 transfection reagent (Invitrogen). Stable clonal cell lines were generated by selection with Zeocin (Invitrogen). One clonal cell line, 3C4-22 was used to generate FVIIIFc for characterization in vivo. Recombinant FVIIIFc was produced and purified (McCue J T, et al., J. Chromatogr. A 2009; 7824-7830, incorporated by reference herein in its entirety) at Biogen Idec (Cambridge, Mass.). The transfection strategy described above was expected to yield three products, i.e., monomeric rFVIIIFc hybrid, dimeric rFVIIIFc hybrid and dimeric Fc. However, there was essentially no dimeric rFVIIIFc detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIIIFc. It is possible that the size of dimeric rFVIIIFc was too great and prevented efficient secretion from the cell. This result was beneficial since it rendered the purification of the monomer less complicated than if all three proteins had been present. The material used in these studies had a specific activity of approximately 9000 IU/mg. In addition, these human cells produced higher protein level than other cells that were attempted in this experiment.

Recombinant FVIII

Recombinant B-domain deleted FVIII (ReFacto®) was purchased from Novis Pharmaceuticals and was prepared according to manufacturer's instructions. ReFacto® (recombinant B-domain deleted FVIII) has the same amino acid sequence as amino acids 1 to 1438 of SEQ ID NO:2.

Hemophilia A animals

The hemophilia A mice are FVIII exon 16 knockouts on a 129×B6 background that were obtained from Dr. Kazazian at the University of Pennsylvania (Bi L, et al., Nat. Genet. 1995; 10(1):119-121, incorporated by reference herein in its entirety) and bred at Syntonix. These mice exhibit prolonged whole blood clotting times (>60 min), and are thus a good model of severe hemophilia A.

Hemophilia A dogs were from the in-bred colony maintained at the Francis Owen Blood Research Laboratory at the University of North Carolina, Chapel Hill (Graham, J B, et al., J. Exp. Med. 1949; 90:97-111, incorporated by reference herein in its entirety). These dogs have a severe hemophilic phenotype comparable to the severe form of the human disease (Graham, J B, et al., J. Exp. Med. 1949; 90:97-111; Lozier, J N, et al., Proc. Natl. Acad. Sci. 2002; 99:12991-12996, each of which is incorporated by reference herein in its entirety).

Study Designs

Hemophilia A Mouse Studies

The effect of rFVIIIFc and ReFacto® on whole blood clotting time (WBCT) was studied in FVIII-deficient mice. Each protein was administered intravenously at 50 IU/kg and blood was collected from the tail vein of each mouse pre-dose and various time points post-dosing. The blood samples were incubated in microtubes at 37° C. and visually inspected once per minute for the presence of a clot. Time of clot formation was recorded. If no clot formed by 60 min, the clotting time was recorded as >60 min. Blood from normal mice clots in approximately 4 min (range 2-7 min, n=10 mice) in the WBCT assay.

In a second set of studies, hemophilia A mice were administered a single intravenous dose of 50 IU/kg rFVIIIFc, ReFacto® or Advate® (4 mice per time point). Blood was collected by cardiac puncture in one tenth volume 3.2% sodium citrate at 0.25, 8, 24, 48 and 72 hr after dosing. Plasma was prepared and stored at −80° C. until analysis for FVIII activity using a FVIII-specific chromogenic activity assay.

Hemophilia A Dog Studies

In a single dose PK/PD study of rFVIIIFc, two hemophilia A dogs from the Chapel Hill colony were administered a single intravenous dose of 125 IU/kg and blood samples were collected pre-dose and after dosing at selected time points for WBCT, activated partial thromboplastin time (aPTT), FVIIIFc plasma concentration, hematology and serum chemistry. Time points for WBCT included pre-dose, 5 and 30 min and 1, 2, 4, 8, 24, 32, 48, 72, 96, 144, and 168 hr after dosing. Blood collections for clotting activity (aPTT) and FVIIIFc plasma concentration included the time points listed above for WBCT as well as 15 min and 3, 6, 12 hours after dosing.

A second study was conducted in which ReFacto® (114 IU/kg for dog M12 and 120 IU/kg for dog M38) was administered intravenously. WBCT was measured until clotting times were ≥20 min (consistent with FVIII:C>1%), and then

125 IU/kg rFVIIIFc was administered intravenously to the same dogs and blood samples were collected for WBCT, aPTT, FVIIIFc plasma concentration, hematology and serum chemistry. Time points for WBCT included pre-dose, 5 and 30 min and 1, 2, 4, 8, 24, 32, 48, 72 hr after dosing. Blood was also collected at 96, 120, 144, and 168 hr after dosing with FVIIIFc. Blood collections for clotting activity and FVIIIFc plasma concentration included the time points listed above for WBCT as well as 15 min and 3, 6, 12 hours after dosing.

The WBCT procedure in hemophilia A dogs was slightly different than that in the hemophilia A mice. After dosing with rFVIIIFc or ReFacto®, one mL of blood was collected at various time points and 0.5 mL was distributed into two siliconized glass tubes which were subsequently placed into a 28° C. water bath. Beginning at one minute, one tube was tilted every 30 sec, the second left undisturbed. When a clot formed in the tilted tube, the second tube was then tilted every 30 sec until a clot formed. The time for a fully gelled clot in the second tube was recorded as the WBCT.

FVIII Activity in Plasma

Measurement of FVIII Activity in Plasma by FVIII-Specific Chromogenic Assay

Plasma samples were tested for FVIII activity by an automated chromogenic method using a Sysmex CA1500 instrument and reagents were from Siemans Healthcare Diagnostics (Dallas, Tex., kit #B4238-40). Activity of rFVIIIFc was determined using a standard curve created using the 7th International Standard Factor FVIII Concentrate (NIBSC code 99/678) spiked into human FVIII-depleted plasma (Stago USA) at concentrations ranging from 1.5-0.016 IU/mL.

Measurement of rFVIIIFc or FVIII by ELISA

FVIIIFc in Dog Plasma by ELISA

A FVIII antibody specific to the A1 domain (Green Mountain Antibodies: GMA-8002) was coated on 96 well plates and incubated for 1 hr at 37° C. The coated plates were blocked with Tris-buffered saline containing Tween 20, $CaCl_2$ and bovine serum albumin for 1 hr at room temperature and then standards, controls and samples that were prepared in normal dog plasma, were diluted 1:10 and then added to the plates and incubated for 1 hour at 37° C. The plates were washed and then donkey $(F(ab)'_2)$ anti-human Fc-HRP (Jackson: 709-036-098) was added and incubated for 1 hr at 37° C. After washing, TMB (BioFx supersensitive substrate: TMBS-0100-01) was added to the plates, the substrate reaction was quenched with acid and absorbance was measured on a SpectraMax Plus plate reader (Molecular Devices) at 450 mm

ReFacto® in Dog Plasma by ELISA

An anti-FVIII antibody specific to the A1 domain on the heavy chain (Green Mountain Antibodies: GMA-8002) was coated on 96 well plates and incubated for 2 hr at room temperature. The coated plates were blocked for 1 hr at 37° C. and after washing, the standards, controls and samples were prepared in normal dog plasma then diluted 1:10 were added to the plates and incubated for 2 hr at room temperature. The plates were washed then treated with the detection antibody, a pre-diluted anti-FVIII horse radish peroxidase conjugate (Affinity Biologicals: F8C-EIA-D), and incubated at room temperature for 1 hr. After washing TMB (BioFx supersensitive substrate: TMBS-0100-01) was added to the plates for 10 min. The substrate reaction was quenched with acid and the signal was measured on a SpectraMax Plus plate reader (Molecular Devices) at a wavelength of 450 nm.

Measurement of Fibrinogen

The concentration of fibrinogen in plasma was measured at Esoterix (Research Triangle Park, N.C.) using a kit that contains HemosIL™ PT-Fibrinogen-HS reagent (Instrumentation Laboratory, Lexington, Mass., Catalog #0008468210) and an ACL 7000 Coagulation Analyzer (Beckman Coulter), according to the manufacturer's instructions.

Measurement of Platelets

Platelets were counted in EDTA anti-coagulated whole blood by automated methods using the Vet-ABC-Diff Hematology Analyzer programmed with a species specific smart card (SCIL Animal Care Co., Gurnee, Ill.).

Pharmacokinetic Analysis

The pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin software from Pharsight, version 5.2 (Mountain View, Calif.). PK parameters included the maximum concentration in plasma (C_{max}), area under the plasma concentration versus time curve (AUC), elimination half-life ($t_{1/2}$), volume of distribution (Vss), and clearance (Cl).

Results

Recombinant FVIII-Fc

rFVIIIFc is a recombinant fusion of human B-domain deleted FVIII with Fc from human IgG1, with no intervening linker sequence (rFVIIIFc; FIG. **1**).

Purified rFVIIIFc had a specific activity of approximately 9000 IU/mg as determined using a chromogenic activity assay. Recombinant B-domain deleted FVIII (ReFacto®) has a reported specific activity of 9110-13700 IU/mg. Conversion of specific activity into IU/nmol to take into account the size difference between FVIIIFc and ReFacto® (216 kDa and 170 kDa respectively), indicates that the two proteins have approximately equivalent specific activities (1970 IU/nmol for rFVIIIFc and 1521-2287 IU/nmol for ReFacto®). Thus the FVIII activity of rFVIIIFc is not affected by fusion of the C-terminus of human FVIII to the N-terminus of human Fc.

Administration to Hemophilia A Mice

A single 50 IU/kg dose of rFVIIIFc or ReFacto® was administered intravenously to FVIII-deficient mice (n=6/group). Blood samples were collected pre-dose and after dosing through 120 hr and WBCT determined as described in Materials and Methods. Baseline WBCT were greater than 60 min. Data from a representative experiment are shown in FIG. **2** and Table 3. Immediately after dosing with either rFVIIIFc or ReFacto®, WBCT was corrected to 2-17 minutes. Blood from mice treated with ReFacto® lost the ability to clot by 42 hr, whereas blood from all mice treated with rFVIIIFc still clotted at 96 hr, the blood from one of six was clotted at 113 hr, but all had lost the ability to clot by 120 hr. These data suggest that the duration of effect for rFVIIIFc is approximately two to three times longer than for ReFacto®.

The chromogenic activity of rFVIIIFc, ReFacto® or Advate® (full-length recombinant FVIII) was studied in the FVIII-deficient mice after a single intravenous dose of 50 IU/kg. Blood was collected pre-dose and after dosing at 8, 24, 48, and 72 hr. The activity was measured using a FVIII-specific chromogenic activity assay and is shown in FIG. **3**. The pharmacokinetic parameters are reported in Table 4. The circulating half-life for rFVIIIFc was approximately 1.6 to 2 fold longer (11.1 hr) compared to Advate® (7 hr) and ReFacto® (5 hr). The Cmax was 1.6±0.36 IU/mL for rFVIIIFc compared to 0.47±0.30 IU/mL for Advate® and 0.67±0.44 IU/mL for ReFacto®. The systemic exposure of rFVIIIFc was markedly greater for rFVIIIFc (22.6 hr-IU/mL) compared to ReFacto® (6.94 hr·IU/mL) and Advate® (3.90 hr·IU/mL) and clearance for rFIIIFc was notably lower (2.09 mL/hr/kg) compared to both ReFacto® (7.2 mL/hr/kg) and Advate® (12.8 hr/mL/kg) in the hemophilia A mice.

Administration to Hemophilia A Dogs

The pharmacodynamics (PD) and pharmacokinetics (PK) of rFVIIIFc were studied in the Chapel Hill colony of hemo-

philia A dogs. A single intravenous dose of 125 IU/kg rFVIIIFc was administered to each of four hemophilia A dogs and the WBCT was immediately corrected to normal (FIG. **4**). The range of WBCT in normal dogs is 8-12 min. The WBCT remained below 20 min, the time consistent with FVIII: C>1%, through approximately 96 hr with the exception of one dog that had WBCT <20 min through 72 hr. In addition, aPTT was also immediately corrected to normal (Table 6). The concentration of rFVIIIFc in plasma was measured using a specific ELISA which was designed to detect both the FVIII and Fc portions of the molecule. The plasma concentration versus time curves are shown in FIG. **5**. PK analysis of the data showed that the t_{10} was 15.7±1.7 hr (Table 5). Similar results were obtained when rFVIIIFc was measured using a FVIII-specific chromogenic activity assay ($t_{1/2}$=15.4±0.3 hr, Table 5) and the plasma concentration versus time curves were similar using both methods (FIGS. **5** and **6**). When the activity data were converted from IU/mL to ng/mL using the specific activity for rFVIIIFc, there was a good correlation with the ELISA data, thereby demonstrating that the protein that was measured by ELISA was fully active.

Two of the dogs treated with rFVIIIFc also received a single dose of ReFacto®, 114 IU/kg for dog M12 and 120 IU/kg for dog M38, 72 hr prior to dosing with rFVIIIFc. WBCT and aPTT were corrected to normal immediately after dosing with ReFacto®. However, the WBCT normalization after the single dose of rFVIIIFc lasted approximately twice as long compared to ReFacto® (FIG. **4**). Moreover, the plasma half-life of rFVIIIFc (15.7±1.7 hr) was approximately twice as long for rFVIIIFc compared to ReFacto® (7.0 and 6.7 hr) when the concentration of the proteins in plasma were measured by ELISA (Table 5). Similar results were obtained when the two molecules were measured by FVIII-specific chromogenic activity.

To assess the potential risk of thrombogenicity, platelets and fibrinogen were measured. After dosing with either rFVIIIFc or ReFacto®, platelet numbers and plasma fibrinogen concentration did not change from pre-dose values (data not shown).

Discussion

Recombinant FVIIIFc was produced in human embryonic kidney 293 (HEK 293) cells from a stably transfected cell line and was purified from cell culture medium. Production in a human cell line represents a significant change in manufacturing compared to currently marketed rFVIII products which are produced in either Chinese Hamster Ovary cells or Baby Hamster Kidney cells. The rationale for this change was that it was expected that the human cells were best equipped to perform the necessary post-translational modifications for the FVIII portion of this molecule.

Conversion of the specific activity to IU/nmol to take into account the difference in molecular weights for rFVIIIFc and recombinant B-domain deleted FVIII (ReFacto®) indicated that the specific activities are similar for both proteins (1970 IU/nmol for rFVIIIFc and 1521-2287 IU/nmol for ReFacto®). It is somewhat surprising that the specific activity for rFVIIIFc is not affected by fusion of the C terminus of FVIII with the N-terminus of Fc since the C1 and C2 domain of FVIII are involved in phospholipid binding which is essential for full FVIII activity (Fay, P J, J. Hematology 83:103-8 (2006) and Raut, S, et al., Br. J. Haematol. 107:323 (1999), each of which is incorporated by reference herein in its entirety).

Treatment of hemophilia A is on-demand at the time of a bleeding episode or by prophylaxis for the prevention of bleeding. Although on-demand treatment is still frequently used, there is a trend toward prophylaxis and the prevention of joint damage (Blanchette P, et al., Haemophilia 2004: 10; 679-683, Manco-Johnson, M J, et al., N. Engl. J. Med. 2007; 357:535-544, each of which is incorporated by reference herein in its entirety). Current FVIII products are administered every two to three days for prophylaxis due to the relatively short half-life of 10-12 hr in order to maintain a FVIII:C above 1% in patients (Morfini, M, Haemophilia 2003; 9 (suppl 1):94-99; discussion 100, White G C, et al., Thromb. Haemost. 1997:77:660-7, Blanchette, P, et al., J. Thromb. Haemost. 2008 August; 6(8):1319-26, each of which is incorporated by reference herein in its entirety). Longer-acting FVIII therapies that provide prolonged protection from bleeding would represent a marked improvement in the quality of life for patients with hemophilia A. Strategies to extend the half-life of clotting factors include those that have been successful for other molecules, including pegylation (Rostin J, et al., Bioconj. Chem. 2000; 11:387-96, incorporated by reference herein in its entirety), glycopegylation (Stennicke H R, et al., Thromb. Haemost. 2008; 100:920-8, incorporated by reference herein in its entirety), formulation with pegylated liposomes (Spira J, et al., Blood 2006; 108: 3668-3673, Pan J, et al., Blood 2009; 114:2802-2811, each of which is incorporated by reference herein in its entirety) and conjugation with albumin (Schulte S., Thromb. Res. 2008; 122 Suppl 4:S14-9, incorporated by reference herein in its entirety). Pegylation represents an approach to reduce clearance, however, the effect of the modification in vivo is currently unknown. The outcome of direct pegylation of FVIII on in vivo is currently unknown, whereas FVIII formulated with pegylated liposomes has been studied clinically and showed a modest to no effect on bleeding periods (Spira J, et al., Blood 2006; 108:3668-3673, Spira J, et al., Thromb. Haemost. 2008 September; 100(3):429-34, each of which is incorporated by reference herein in its entirety).

The present approach to extend the half-life of FVIII was to recombinantly fuse FVIII to the Fc domain of IgG1. Fc binds to the naturally occurring receptor, FcRn, of which the normal function is protection of IgG from degradation. The results described herein represent the initial pharmacokinetic and efficacy characterization of rFVIIIFc compared to a rFVIII product in hemophilia A mice and hemophilia A dogs. In both species, the half-life of rFVIIIFc was approximately twice that of rFVIII when measured by FVIII activity or ELISA (dogs only). These data also correlated well with the WBCT results from both animal models, i.e. the duration of the effect of rFVIIIFc on WBCT was approximately twice as long compared to ReFacto®. In dogs, the C_{max} and clearance were similar for rFVIIIFc and ReFacto®, but the AUC and volume of distribution at steady state were approximately 1.5 fold and 2 fold greater for rFVIIIFc compared to ReFacto®, respectively. The PK parameters for ReFacto® in this animal model are consistent with the values reported in the literature (Brinkhous K, et al., Sem. Thromb. Haemost. 2002; 28:269-272, incorporated by reference herein in its entirety).

If these findings translate to the same extension of half-life in humans, this could represent a significant advancement in the treatment of patients with hemophilia A.

Additional References (each of which is incorporated herein by reference in its entirety)

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Example 2

The objective of the study was to determine the pharmacokinetics and pharmacodynamics of rFVIIIFc and BDD-rFVIII (Xyntha®) in cynomolgus monkeys after a single intravenous dose.

Materials and Methods

rFVIIIFc (Biogen Idec), supplied as a frozen liquid at a concentration of 1.2 mg/mL, and 9882 IU/mL. The specific activity is 8235 IU/mg. Storage was at −70° C. It was diluted prior to injection.

Name: Xyntha (Novis Pharmaceuticals), Supplied as a lyophilized powder which was reconstituted according to the manufacturer's instructions to produce a solution with a nominal concentration of 525 IU/mL. Storage was according to the manufacturer's recommendations.

Animals

Cynomolgus monkeys from the New Iberia Research Center (NIRC) colony were used, and the study (NIRC Study #8733-0903) was conducted under an approved NIRC IACUC protocol (APS 2008-8733-058) at NIRC in New Iberia, La.

Six naïve cynomolgus monkeys (three males, three females) that were determined to be in good health were used in the study.

The study was performed in compliance with the protocol and UL Lafayette-NIRC Standard Operating Procedures.

Study Design

rFVIIIFc was administered intravenously at 125 IU/kg to each of six monkeys (three males, three females). Xyntha (BDD-rFVIII) was administered intravenously to the same animals at 125 IU/kg in a crossover design. Group 1 animals (n=3) received Xyntha on Day 0 and rFVIIIFc on Day 3, while Group 2 animals (n=3) received rFVIIIFc on Day 0 followed by Xyntha on Day 4. The additional day between doses for group 2 was to ensure that the rFVIIIFc had sufficient time to decrease below projected baseline levels. Blood was collected for plasma in one-tenth volume 3.2% sodium citrate from each animal predose and after dosing at 0.25, 4, 12, 24, 36, 48 and 72 hr for measurement of rFVIIIFc or Xyntha by ELISA and a FVIII-specific chromogenic activity assay.

ELISA to Measure rFVIIIFc and FVIII in Plasma

Method to Measure rFVIIIFc in Monkey Plasma

This Enzyme Linked ImmunoSorbent Assay (ELISA) is designed to quantify rFVIIIFc in monkey plasma. In this ELISA method, goat anti-human IgG-(H+L) antibody (monkey absorbed) from Bethyl Laboratories (Cat#A80-319A) is diluted in Coating Buffer and immobilized onto a 96-well microtiter sample plate. The plate is aspirated, and all unadsorbed sites are blocked with the addition of Blocking Buffer (3% BSA/1×Tris) for approximately 2 hours at 37° C. Plasma samples are diluted 1:20 with High Calcium Sample Dilution Buffer (3% Non-Fat Dry Milk/TBST with 30 mM $CaCl_2$) and dispensed onto the sample plate. Plates are incubated for approximately 2 hours at 37° C. The plate is subsequently washed and mouse anti-B domain-deleted (α.B-DDA1) Factor VIII (A1 domain) antibody from Green Mountain Antibodies (Cat#GMA-8002) is added to the plate and incubated for approximately 1 hour at 37° C. After washing the plate, HRP-conjugated goat anti-mouse IgG2a antibody from Southern Biotech (Cat#1080-05) is added to the plate and incubated for approximately 30 minutes at room temperature. The plate is washed again and a tetramethylbenzidine (TMB) peroxidase substrate solution is added and incubated for approximately 30 minutes at room temperature. The reaction is stopped by addition of a non-acidic Stop Solution. Color develops in proportion to the amount of rFVIIIFc in the sample. Plates are read on an absorbance plate reader using a single detection wavelength, 650 nm. rFVIIIFc concentrations are determined on a standard curve obtained by plotting optical density (OD) versus concentration using a four-parameter logistic curve-fitting program. The calibration curve range of this method is 0.400 ng/mL-51.2 ng/mL in 5% monkey plasma (8.00 ng/mL-1024 ng/mL in 100% monkey plasma). One calibrator outside the qualified range of the assay at 0.200 ng/mL in 5% monkey plasma may be included to serve as an anchor point to facilitate curve-fitting. The anchor point is removed or retained based on the best fit of the curve (i.e., the highest number of standards read within defined accuracy, % RE).

Method to Measure FVIII in Monkey Plasma

This Enzyme Linked ImmunoSorbent Assay (ELISA) is designed to quantify FVIII in monkey plasma. In this ELISA method, mouse αBDDA1 FVIII antibody from Green Mountain Antibodies (Cat# GMA-8002) is diluted in Coating Buffer and immobilized onto a 96-well microtiter sample plate. The plate is aspirated, and all un-adsorbed sites are blocked with the addition of Blocking Buffer (3% BST/1× Tris) for approximately 1 hour at 37° C. Plasma samples are diluted 1:20 with High Calcium Sample Dilution Buffer (Blocking Buffer with 100 mM CaCl2) and dispensed onto the sample plate. Plates are incubated for approximately 2 hours at 37° C. After washing the plate, a Detecting Antibody from the Affinity Biologicals Kit, an HRP labeled polyclonal antibody (Cat#F8C-EIA-D), is further diluted in TBS/0.05% Tween 20, and added to the plate and incubated for approximately 1 hour at room temperature. The plate is washed again and a tetramethylbenzidine (TMB) peroxidase substrate solution is added and incubated for approximately 30 minutes at room temperature. The reaction is stopped by addition acidic Stop Solution. Color develops in proportion to the amount of FVIIIFc in the sample. Plates are read on an absorbance plate reader using a single detection wavelength, 450 nm. FVIII concentrations are determined on a standard curve obtained by plotting optical density (OD) versus concentration using a four-parameter logistic curve-fitting program. The calibration curve range of this method is 0.625 ng/mL-20 ng/mL in 5% monkey plasma (12.5 ng/mL-400 ng/mL in 100% monkey plasma). Two calibrators outside the qualified range of the assay at 0.313 and 0.156 ng/mL in 5% monkey plasma may be included to serve as anchor points to facilitate curve-fitting. The anchor points can be removed or retained based on the best fit of the curve (i.e., the highest number of standards read within defined accuracy, % RE).

FVIII-Specific Chromogenic Assay

FVIII activity in cynomolgus monkey plasma samples was estimated based on administered dose, and then diluted to approximately 0.25-1 IU/ml in human FVIII-depleted plasma (Diagnostica Stago). Samples were analyzed in a Sysmex CA1500 (Siemens Diagnostic Healthcare) using a FVIII chromogenic kit (Siemens). In this chromogenic assay, rFVIIIFc in the plasma samples is activated by thrombin. Activated Factor VIII (FVIIIa) then accelerates the conversion of Factor X (FX) to Factor Xa (FXa) in the presence of activated Factor IX (FIXa), phospholipids (PL) and calcium

ions. The FXa activity is assessed by hydrolysis of a p-nitroanilide substrate specific to FXa. The initial rate of release of p-nitroaniline (pNA) measured at 405 nm is proportional to the FXa activity, and thus to the FVIII activity in the sample. The limit of quantitation of FVIII activity due to rFVIIIFc in this assay is ~0.3 IU/ml. The assay can measure total FVIII activity down to a lower limit of approximately 0.06 IU/ml with an accuracy of ±20%. The calculated activity of the pre-dose sample for individual animals was subtracted from the value at each time point to generate the PD curves (FVIII activity vs. time).

A standard curve was generated from the NIBSC 7th International Standard FVIII concentrate diluted to 1 1 U/ml in human FVIII-deficient plasma. Standard curves were diluted serially in the Sysmex instrument to yield concentrations of 0.15, 0.1, 0.05, 0.025, 0.0053 and 0.0026 IU/ml. Since the instrument dilutes all samples 1:10 internally, the FVIII standard concentrations correspond to plasma concentrations of 1.5-0.026 IU/ml, which is the range of FVIII activities that can be measured.

PK Analysis

The concentration time profiles were evaluated using the non-compartmental analysis module in the WinNonlin software program (Version 5.2, Pharsight Corporation, Mountain View, Calif.).

Results

The concentration of rFVIIIFc in monkey plasma was measured using a sandwich

ELISA format that measured both the FVIII and Fc portions of the molecule and the data are reported in Table 7. All predose samples were below the limit of quantitation. FIG. **7** illustrates the group mean rFVIIIFc and Xyntha plasma concentrations over time and individual plasma concentration versus time curves are shown in FIG. **8**. A summary of the PK parameters for rFVIIIFc and Xyntha are shown in Tables 9 and 10, respectively. The mean t½ for rFVIIIFc was 11.9±1.7 hr (range 9.3 to 14.1 hr) and for Xyntha, the mean elimination t½ was 12.7*±4.4 hr (range 9.2 to 19.9 hr).

FVIII activity was measured using a FVIII-specific chromogenic activity assay and the data are reported in Table 8. Pre-dose activity due to endogenous FVIII was subtracted from all samples. A graph of the mean group data is shown in FIG. **9** and the individual plasma concentration vs. time curves are shown in FIG. **10**. A summary of the PK parameters are reported for rFVIIIFc and Xyntha in Tables 9 and 10, respectively. The mean elimination t½ was 16.1±6.9 hr (range 11.6 to 29.4 hr) for rFVIIIFc and 12.5±1.7 hr (range 10.4 to 14.3 hr) for Xyntha.

Discussion and Conclusions

The elimination half-lives were similar for rFVIIIFc and Xyntha after a single intravenous dose of 125 IU/kg. whether the test article was measured by ELISA or a chromogenic activity assay.

Example 3

This will be a Phase I/IIa, open-label, crossover, dose-escalation, multi-center, and first-in-human study designed to evaluate the safety, tolerability, and pharmacokinetics of a single dose of rFVIIIFc in subjects with severe (defined as <1 IU/dL [1%] endogenous factor VIII [FVIII]) hemophilia A. A total of approximately 12 previously treated patients will be enrolled and dosed with rFVIIIFc at 25 or 65 IU/kg. After the screening (scheduled within 28 days prior to the first dose of the Advate® [rFVIII], the reference comparator agent) and a minimum of 4-days (96 hours) elapsing with no FVIII treatment prior to the first injection, approximately 6 subjects will receive a single 25 IU/kg dose of Advate® followed by a 3-day (72 hours) pharmacokinetic (PK) profile then crossover and receive a 25 IU/kg single, open-label dose of rFVIIIFc for a 7-day (168 hours) PK profiling. The first 3 subjects will be dosed sequentially. For the first three (3) subjects dosed with 25 IU/kg of rFVIIIFc, each subject will undergo an inhibitor assessment at 14-days (336 hours) post-injection of rFVIIIFc. Dosing of the next subject (for the first three subjects only) will occur once the inhibitor testing is completed. After the 3rd subject completed the 14 day inhibitor assessment, the remaining three subjects at 25 IU/kg and the six subjects at 65 IU/kg will begin enrollment sequentially at least 1 day apart within each dose group.

One week after the last subject receives the 25 IU/kg dose of the rFVIIIFc, approximately 6 unique subjects will be recruited for the 65 IU/kg cohort. Each subject in the 65 IU/kg cohort will receive a single 65 IU/kg dose of Advate® followed by a 4-day (96 hours) PK profiling then crossover and receive a 65 IU/kg single, open-label dose of rFVIIIFc for a 10-day (240 hours) profiling. If a bleeding episode occurs before the first injection of rFVIIIFc in any cohort, subject's pre-study FVIII product should be used for treatment and an interval of at least 4 days must then pass before receiving the first injection of rFVIIIFc for the PK profile.

All subjects will be followed for a 14-day (336 hours) and 28 day safety evaluation period after administration of rFVIIIFc 25 IU/kg or 65 IU/kg for safety. All subjects will undergo pharmacokinetic sampling pre- and post-dosing along with blood samples for analysis of FVIII activity at designated time points.

Example 4

Activity within the Xase Complex

To investigate the binding of the FVIII proteins (rBDD FVIII and rFVIIIFc) with FIXa, and measure the ability of these proteins to activate FX, kinetic studies were performed examining these interactions in the context of the Xase complex. This assay involved the formation of the Xase complex with activated FIX and activated rBDD FVIII or rFVIIIFc protein on a phospholipid surface in the presence of calcium, and monitoring the conversion of FX to FXa as measured by cleavage of a chromogenic or fluorogenic substrate.

Briefly, FVIII is first activated with α-thrombin for 5 min, then mixed with FIXa in the presence of Ca2+, and synthetic phospholipid vesicles (25% phosphatidylserine (PS)/75% phosphatidylcholine (PC)) or platelets. Under conditions described below, FVIIIa and FIXa interact in the presence of a phospholipid surface and calcium ions to form an active Xase complex that mediates the conversion of FX into FXa through proteolytic processing. In turn, FXa cleaves a FXa-specific chromogenic or fluorogenic substrate. The cleaved substrate is chromogenic and therefore the amount of cleaved substrate in a solution is indicative of the amount of FXa generated. This is quantitated by measuring the absorbance of the solution at 405 nm.

A. Activation of Factor X

The ability of rBDD FVIII and rFVIIIFc to activate FX were studied in the context of the Xase complex as described above. Thrombin-activated F VIII proteins were incubated with FIXa and phospholipids in the presence of calcium, then added to different concentrations of FX in the presence of a FX-specific substrate and the rates of FXa generation determined (FIG. **11**).

Based on these data, the Km and Vmax for the different FVIII proteins in the context of the Xase complex were calculated (Chang 1997) (Table 11). Data are expressed as the

mean of six analyses (3 experiments containing duplicate runs)±the corresponding standard deviation. Based on these data, these proteins (rBDD FVIII and rFVIIIFc) were found to have comparable Km and Vmax values, within the variation of the assay. Therefore, the Xase complex formed with rFVIIIFc behaves similarly to the Xase complex formed with the licensed product rBDD FVIII (ReFacto) with respect to interactions with phospholipids and ability to activate FX. Note that these comparable data also demonstrate that rFVIIIFc is activated to a comparable degree as rBDD FVIII after a short incubation with thrombin.

B. Interaction with FIXa

The interaction between rBDD FVIII and rFVIIIFc with FIXa were also examined in the context of the Xase complex. The Xase complex was assembled as above, using a fixed amount of FX and varying FIXa levels, and FXa generation rates determined (FIG. **12**). From these data, the Kd value for the Xase complex fixated with both of the FVIII proteins to FIXa were determined (Chang 1997). Data are expressed as the mean of six analyses (3 experiments containing duplicate runs)±the corresponding standard deviation (Table 12). Both proteins were found to have similar Kd and Vmax values, indicating that rFVIIIFc has comparable interactions with FIXa as the licensed rBDD FVIII product.

Example 5

Interim pharmacokinetic data for the Phase I/IIa clinical trial discussed in Example 3 demonstrated the following results for FVIIIFc. FVIIIFc had about a 50% increase in systemic exposure (AUC_{INF}), about 50% reduction in clearance (Cl), and about 50-70% increase in elimination half-life and MRT compared to ADVATE (full length rFVIII). In addition, FVIIIFc showed increased C168, TBLP1, TBLP3, and TBLP5 values compared to ADVATE.

AUC_{INF} Area under the concentration-time curve from zero to infinity

Beta HL Elimination phase half-life; also referred to as $t_{1/2\beta}$

C168 Estimated FVIIIFc activity above baseline at approximately 168 h after dose

Cl Clearance

MRT Mean residence time

TBLP1 Model-predicted time after dose when FVIIIFc activity has declined to approximately 1 IU/dL above baseline

TBLP3 Model-predicted time after dose when FVIIIFc activity has declined to approximately 3 IU/dL above baseline

TBLP5 Model-predicted time after dose when FVIIIFc activity has declined to approximately 5 IU/dL above baseline

Example 6

A recombinant B-domain-deleted factor VIII-Fc (rFVIIIFc) fusion protein has been created as an approach to extend the half-life of FVIII. The pharmacokinetics (PK) of rFVIIIFc were compared to rFVIII in hemophilia A mice. We found that the terminal half-life was twice as long for rFVIIIFc compared to rFVIII. In order to confirm that the underlying mechanism for the extension of half-life was due to the protection of rFVIIIFc by FcRn, the PK were evaluated in FcRn knockout and human FcRn transgenic mice. A single intravenous dose (125 IU/kg) was administered and the plasma concentration measured using a chromogenic activity assay. The Cmax was similar between rFVIIIFc and rFVIII (XYNTHA®) in both mouse strains. However, while the half-life for rFVIIIFc was comparable to that of rFVIII in the FcRn knockout mice, the half-life for rFVIIIFc was extended to approximately twice longer than that for rFVIII in the hFcRn transgenic mice. These results confirm that FcRn mediates or is responsible for the prolonged half-life of rFVIIIFc compared to rFVIII. Since hemostasis in whole blood measured by rotation thromboelastometry (ROTEM) has been shown to correlate with the efficacy of coagulation factors in bleeding models of hemophilia mice as well as in clinical applications, we sought to evaluate the ex vivo efficacy of rFVIIIFc in the hemophilia A mice using ROTEM. Hemophilia A mice were administered a single intravenous dose of 50 IU/kg rFVIIIFc, XYNTHA® (FVIII) or ADVATE® (FVIII). At 5 minutes post dose, clot formation was similar with respect to clotting time (CT), clot formation time (CFT) and α-angle. However, rFVIIIFc showed significantly improved CT at 72 and 96 hr post dose, and CFT and α-angle were also improved at 96 hrs compared to both XYNTHA® (FVIII) and ADVATE® (FVIII), consistent with prolonged PK of rFVIIIFc. Therefore construction of an Fc fusion of FVIII produces a molecule with a defined mechanism of action that has an increased half-life and the potential to provide prolonged protection from bleeding.

Example 7

This Example presents final analysis results for FVIII activity from 16 patients treated with 25 and 65 IU/kg FVIII products. See Examples 3 and 5.

In this Example, rFVIIIFc is a recombinant fusion protein comprised of a single molecule of recombinant B-domain deleted human FVIII (BDD-rFVIII) fused to the dimeric Fc domain of the human IgG1, with no intervening linker sequence. This protein construct is also referred to herein as rFVIIIFc heterodimeric hybrid protein, FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc monomer-dimer. See Example 1, FIG. **1**, and Table 2A.

Preclinical studies with rFVIIIFc have shown an approximately 2-fold prolongation of the half-life of rFVIII activity compared to commercially available rFVIII products. The rationale for this study was to evaluate the safety and tolerability of a single dose of rFVIIIFc in frozen liquid formulation and provide data on the PK in severe hemophilia A subjects. For this study, 16 evaluable subjects were available for PK evaluation. Single administration of two doses of both rFVIIIFc and Advate at a nominal dose of 25 (n=6) and 65 IU/kg of body weight (n=10) were infused intravenously over approximately 10 minutes. Blood samples for plasma PK assessments were obtained before infusion, as well as up to 10 days after dosing. The PK of FVIII activity for both Advate and rFVIIIFc were characterized in this study using a model-dependent method.

Objectives

The primary objective of this study was to assess the safety and tolerability of single administration of two doses of rFVIIIFc (25 and 65 IU/kg) in previously treated patients (PTPs) aged 12 and above with severe hemophilia A.

The secondary objectives were to determine the pharmacokinetics (PK) parameters determined by pharmacodynamic (PD) activity of FVIII over time after a single administration of 25 or 65 IU/kg of rFVIIIFc compared to Advate in one-stage clotting and chromogenic assays.

Study Design (See Example 3)

Blood samples were collected for FVIII activity PK evaluations at the screening visit (within 28 days prior to dosing Advate); on Day 0 (injection of Advate) pre-injection and at 10 and 30 minutes and 1, 3, 6, and 9 hours post-injection; on Day 1 at 24 hours post-injection of Advate; on Day 2 at 48 hours post-injection of Advate; on Day 3 at 72 hours post-

injection of Advate; and on Day 4 at 96 hours post-injection of high dose of Advate (Cohort B only).

Blood samples were collected for FVIII activity PK evaluations on the day of rFVIIIFc injection just prior to the administration of rFVIIIFc, at 10 and 30 minutes and 1, 3, 6, and 9 hours post-injection of rFVIIIFc; on Day 1 at 24 hours post-injection of rFVIIIFc; on Days 2 through 5 at 48, 72, 96, and 120 hours post-injection of rFVIIIFc; on Day 7 at 168 hours post-injection of rFVIIIFc; on Days 8, 9, and 10 at 192, 216, and 240 hours post-injection of high dose of rFVIIIFc (Cohort B only). FVIII activity was also measured at the final study visit (28 days post-injection of rFVIIIFc) at 672 hours post-injection of rFVIIIFc.

Pharmacokinetic Modeling and Calculations

Abbreviations

TBLP1=Model-predicted time after dose when FVIII activity has declined to approximately 1 IU/dL above baseline,

TBLP3=Model-predicted time after dose when FVIII activity has declined to approximately 3 IU/dL above baseline

KV_M=Cmax_M/Actual Dose (IU/kg)

KV_OB=Cmax_OB/Actual Dose (IU/kg)

IVR_M=100×Cmax_M×Plasma Volume (dL)/Total Dose in IU; where plasma volume in mL=(23.7×Ht in cm)+(9.0×Wt in kg)−1709.

IVR_OB=100×Cmax_OB×Plasma Volume (dL)/Total Dose in R1; where plasma volume in mL=(23.7×Ht in cm)+(9.0×Wt in kg)−1709.

Results

FIG. **13**. Observed group mean (±SE) FVIII activity versus time profiles, sorted by dose level, grouped by compound (one-stage assay, 25 IU/kg (A) and 65 IU/kg (B)) and (chromogenic assay, 25 IU/kg (C) and 65 IU/kg (D)).

FIG. **14**. Observed group mean (±SE) FVIII activity versus time profiles, grouped by dose level and compound (one-stage assay; A) (chromogenic assay; B).

Single-Dose Pharmacokinetics (One-Stage Assay)

Observed FVIII activity increased sharply after the short IV infusion of either Advate or rFVIIIFc, with mean (±SD) model-predicted Cmax values of 56.6±4.74 and 121±28.2 IU/dL for Advate and 55.6±8.18 and 108±16.9 IU/dL for rFVIIIFc for the and 65 IU/kg dose groups, respectively. All Advate- and rFVIIIFc-treated patients had dose-related increases in FVIII activity. The observed increase in both Cmax and AUCINF was slightly less than proportional to dose over the dose range evaluated.

After the end of the infusion, the decline of the observed FVIII activity exhibited monoexponential decay characteristics until the baseline level was reached. The rate of decline in FVIII activity was slower for rFVIIIFc than for Advate with mean (±SD) model-predicted elimination half-life values of 11.9±2.98 and 10.4±3.03 hr for Advate and 18.0±3.88 and 18.4±6.99 hr for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Elimination half-life values appeared to be dose-independent over the dose range evaluated for both FVIII products.

Total systemic FVIII exposure (assessed by AUCINF) was 48% and 61% greater following rFVIIIFc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (±SD) model-predicted AUCINF values were 974±259 and 1810±606 hr*IU/dL for Advate and 1440±316 and 2910±1320 hr*IU/dL for rFVIIIFc for the and 65 IU/kg dose groups, respectively.

Similar to elimination half-life, the MRT was prolonged for rFVIIIFc relative to Advate. Mean (±SD) model-predicted MRT values were 17.1±4.29 and 14.9±4.38 hr for Advate and 25.9±5.60 and 26.5±10.1 hr for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. MRT values appeared to be dose-independent over the dose range evaluated for both FVIII products.

In addition, primary PK parameter values for CL and V were determined. CL values for rFVIIIFc only accounted for ~66% of those observed for Advate at equivalent doses. Mean (±SD) model-predicted CL values were 2.70±0.729 and 4.08±1.69 mL/hr/kg for Advate and 1.80±0.409 and 2.69±1.25 mL/hr/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIIIFc with mean (±SD) model-predicted V values of 43.9±4.27 and 56.1±13.4 mL/kg for Advate and 45.3±7.23 and 61.6±10.6 mL/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Slight increases in mean CL and V values were noted with increasing dose of Advate and rFVIIIFc; however, the increase in standard deviations at the 65 IU/kg dose coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters. For example, the CV % geometric mean CL value for the rFVIIIFc treatment group increased from 23.0% (25 IU/kg) to 48.6% (65 IU/kg).

In addition to the primary PK parameters, secondary PK parameters (e.g. K-values, IVR, etc.) were determined to evaluate FVIII duration of effect. Evidence of PK difference was also observed with rFVIIIFc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc appeared to be comparable. A slight increase in TBLP1 and TBLP3 values were observed with increasing dose of Advate and rFVIIIFc. In contrast, slight decreases in mean IVR and K-values were noted with increasing dose of Advate and rFVIIIFc. As previously indicated, an assessment of the dose dependency of these parameters is confounded by limited dose levels.

Mean (±SD) observed TBLP1 were 2.88±0.733 and 2.93±0.848 IU/dL per IU/kg for Advate and 4.28±0.873 and 5.16±2.02 IU/dL per IU/kg for rFVIIIFc for the and 65 IU/kg dose groups, respectively. Mean (±SD) observed TBLP3 were 2.06±0.527 and 2.26±0.666 IU/dL per IU/kg for Advate and 3.09±0.623 and 3.93±1.59 IU/dL per IU/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

Mean IVR and K-values calculated using observed Cmax values (subtracted with baseline and residual drug within the model) were generally greater than values determined using model-predicted Cmax values; consistent with slight underestimation of the observed peak activity using the one-compartment model. Mean (±SD) observed K-values were 2.57±0.198 and 2.13±0.598 IU/dL per IU/kg for Advate and 2.46±0.330 and 1.85±0.332 IU/dL per IU/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (±SD) observed IVR values were 94.1±15.6 and 85.8±16.5% for Advate and 89.5±11.9 and 74.8±6.72% for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

Single-Dose Pharmacokinetics (Chromogenic Assay)

Observed FVIII activity increased sharply after the short IV infusion of either Advate or rFVIIIFc, with mean (±SD) model-predicted Cmax values of 70.2±9.60 and 157±38.6 IU/dL for Advate and 70.3±10.0 and 158±34.7 IU/dL for rFVIIIFc for the and 65 IU/kg dose groups, respectively.

All Advate- and rFVIIIFc-treated patients had dose-related increases in FVIII activity. The observed increase in both Cmax and AUCINF was slightly less than proportional to dose over the dose range evaluated.

After the end of the infusion, the decline of the observed FVIII activity exhibited monoexponential decay characteristics until the baseline level was reached. The rate of decline in FVIII activity was slower for rFVIIIFc than for Advate with mean (±SD) model-predicted elimination half-life values of

10.7±1.98 and 10.3±3.27 hr for Advate and 16.2±2.92 and 19.0±7.94 hr for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Elimination half-life values appeared to be dose-independent over the dose range evaluated for both FVIII products.

Total systemic FVIII exposure (assessed by AUCINF) was ~53% and 84% greater following rFVIIIFc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (±SD) model-predicted AUCINF values were 1080±236 and 2320±784 hr*IU/dL for Advate and 1650±408 and 4280±1860 hr*IU/dL for rFVIIIFc for the and 65 IU/kg dose groups, respectively.

Similar to elimination half-life, the MRT was prolonged for rFVIIIFc relative to Advate. Mean (±SD) model-predicted MRT values were 15.3±2.86 and 14.8±4.72 hr for Advate and 23.4±4.22 and 27.3±11.4 hr for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. MRT values appeared to be dose-independent over the dose range evaluated for both FVIII products.

In addition, primary PK parameter values for CL and V were determined. CL values for rFVIIIFc only accounted for ~58-66% of those observed for Advate at equivalent doses. Mean (±SD) model-predicted CL values were 2.39±0.527 and 3.21±1.40 mL/hr/kg for Advate and 1.57±0.349 and 1.86±0.970 mL/hr/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIIIFc with mean (±SD) model-predicted V values of 35.8±5.52 and 43.6±11.2 mL/kg for Advate and 35.9±6.65 and 42.7±8.91 mL/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Increases in mean CL and V values were noted with increasing dose of Advate and rFVIIIFc; however, the increase in standard deviations at 65 IU/kg coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters.

In addition to the primary PK parameters, secondary PK parameters (e.g. K-values, IVR, etc.) were determined to evaluate FVIII duration of effect. Evidence of PK difference was also observed with rFVIIIFc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc appeared to be comparable.

A slight increase in TBLP1 and TBLP3 values were observed with increasing dose of Advate and rFVIIIFc. In contrast, slight decreases in mean IVR and K-values were noted with increasing dose of Advate and rFVIIIFc. As previously indicated, an assessment of the dose dependency of these parameters is confounded by limited dose levels.

Mean (±SD) observed TBLP1 were 2.70±0.511 and 3.09±0.978 IU/dL per IU/kg for Advate and 4.06*0.798 and 5.66±2.38 IU/dL per IU/kg for rFVIIIFc for the and 65 IU/kg dose groups, respectively. Mean (±SD) observed TBLP3 were 1.98±0.377 and 2.39±0.718 IU/dL per IU/kg for Advate and 3.04±0.598 and 4.44±1.84 IU/dL per IU/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

Mean IVR and K-values calculated using observed Cmax values (subtracted with baseline and residual drug within the model) were generally greater than values determined using model-predicted Cmax values; consistent with slight underestimation of the observed peak activity using the one-compartment model. Mean (±SD) observed K-values were 3.08±0.429 and 2.85±0.721 IU/dL per IU/kg for Advate and 3.12±0.451 and 2.92±0.985 IU/dL per IU/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (*SD) observed IVR values were 112±14.5 and 116±26.9% for Advate and 113±16.3 and 117±33.6% for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

Conclusions

All Advate- and rFVIIIFc-treated patients had comparable dose-related increases in Cmax and AUCINF over the dose range evaluated. Peak plasma levels of Advate and rFVIIIFc activity were generally observed within the first hour after the end of the infusion and remained detectable for several days after dosing. After the end of infusion, the decline in baseline corrected FVIII activity exhibited monoexponential decay until the baseline was reached for both products. Parameter values for elimination half-life and MRT appeared to be dose-independent over the dose range evaluated for both FVIII products. Slight increases in mean CL and V values were noted with increasing dose of Advate and rFVIIIFc; however, increased intersubject variability at the 65 IU/kg coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters.

Comparison of rFVIIIFc and Advate activity PK revealed an approximate 48-61% (One-Stage Assay) or 53-84% (Chromogenic Assay) increase in systemic exposure, approximate 30-40% reduction in clearance, and an approximate 50-80% increase in both elimination half-life and MRT for rFVIIIFc relative to Advate at comparable doses. Evidence of PK difference was also observed with rFVIIIFc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc appeared to be comparable.

The PK parameters obtained from Chromogenic Assay results generally agreed with those from the One-Stage Assay, except that the Chomogenic Assay yielded a higher estimation of exposure parameters (e.g. Cmax, AUCINF, etc.).

With the observed improvements in PK, rFVIIIFc may provide a prolonged protection from bleeding, allowing less frequent injections for individuals with Hemophilia A.

Example 8

On the basis of the interim PK analysis from the first-inhuman study of rFVIII:Fc (Example 3), the A-LONG study was designed. A-LONG is an open label, multi-center evaluation of the safety, pharmacokinetics, and efficacy of recombinant Factor VIII Fc fusion (FVIII:Fc) in the prevention and treatment of bleeding in previously treated subjects with severe hemophilia A (defined as <1 IU/dL [<1%] endogenous FVIII).

Approximately 106 subjects will be enrolled into one of three regimens: a tailored prophylaxis regimen (arm 1), a weekly dosing regimen (arm 2), and an on-demand regimen (arm 3).

Arm 1: Tailored Prophylaxis Regimen

Arm 1 will include an overall group and a PK subgroup. Approximately 66 subjects will be enrolled. The initial regimen will be twice weekly at 25 IU/kg on the first day, followed by 50 IU/kg on the fourth day of the week. Subjects will administer rFVIIIFc on this weekly prophylaxis regimen until PK results for rFVIIIFc are available. Base don these results, a tailored prophylaxis regimen will be established for each individual, in which the dose and interval will be determined to maintain a trough level of 1-3% FVIII activity. Each subject will then administer his individually tailored prophylaxis regimen throughout the study.

Subjects will be monitored throughout the study and ongoing dose and interval adjustments will be made. Adjustments will only be made when a subject experiences unacceptable bleeding episodes defined as ≥2 spontaneous bleeding episodes over a rolling two-month period. In this case, adjustment will target trough levels of 3-5%.

Arm 2: Weekly Dosing Regimen

Approximately 20 subjects will be enrolled/randomized and undergo abbreviated rFVIIIFc PK profiling as follows: Washout of at least 96 hours; a single dose of rFVIIIFc 65 IU/kg; Abbreviated sampling beginning on rFVIIIFc Day 0, including pre-injection and 10 (±2) minutes, 3 hours (±15 minutes), 72 (±2) hours [Day 3], and 96 (±2) hours [Day 4] from the start of injection. Following the abbreviated PK profiling, subjects will then administer a fixed dose of 65 IU/kg rFVIIIFc every 7 days.

Arm 3: On-Demand Regimen

A minimum of 10 major surgeries in at least 5 subjects will be evaluated in the study. Major surgery is defined as any surgical procedure (elective or emergent) that involves general anesthesia and/or respiratory assistance in which a major body cavity is penetrated and exposed, or for which a substantial impairment of physical or physiological functions is produced (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation).

For prophylaxis during surgery, subjects will be treated with 35 to 50 IU/kg rFVIIIFc every 12 to 24 hours. Prior to surgery, the physician will review the subject's rFVIIIFc PK profile and assess the dose regimen of Factor VIII replacement generally required for the type of planned surgery and the clinical status of the subject. Recommendation for the appropriate dosing of rFVIIIFc in the surgical treatment period, including any rehabilitation time, will take these factors into consideration.

The primary objectives of this study are (a) to evaluate the safety and tolerability of rFVIIIFc administered as prophylaxis, on-demand, and surgical treatment regimens; and (b) to evaluate the efficacy of rFVIIIFc administered as prophylaxis, on-demand, and surgical treatment regimens. The secondary objectives of this study are (a) to characterize the PK profile of rFVIIIFc and compare the PK of FVIIIFc with the currently marketed product, ADVATE; (b) to evaluate individual responses with FVIIIFc; and (c) to evaluate FVIIIFc consumption.

Primary Objectives

- To evaluate safety and tolerability of rFVIIIFc administered as prophylaxis, weekly, on-demand, and surgical treatment regimens
- To evaluate the efficacy of rFVIIIFc administered as tailored prophylaxis, on-demand, and surgical treatment regimens

Secondary Objectives

- To characterize the PK profile of rFVIIIFc and compare the PK of rFVIIIFc with the currently marketed product, Advate®
- To evaluate individual responses with rFVIIIFc
- To characterize the range of dose and schedules required to adequately prevent bleeding in a prophylaxis regimen; maintain homeostasis in a surgical setting; or to treat bleeding episodes in an on-demand, weekly treatment, or prophylaxis setting
- To evaluate rFVIIIFc consumption (e.g., total annualized rFVIIIFc consumption per subject)

Example 9

Clinical ROTEM Assessment

In the study in Example 8, in addition to the measurement of plasma FVIII activity by one-stage activated partial thromboplastin time (aPTT) assay, whole blood rotational thromboelastometry (ROTEM) has also been explored to assess the improvement in global hemostasis by rFVIIIFc and Advate in 2 subjects, specifically, 1 in the low dose cohort and 1 in the high dose cohort.

rFVIIIFc and Advate appear to be comparably active in clot formation when spiked into subjects' blood prior to rFVIIIFc treatment. The clotting time (CT) was linear with respect to the dose of rFVIIIFc and Advate in the range of approximately 1% of 100% of normal, and the dose response was comparable between rFVIIIFc and Advate in the same subject.

Following dosing with Advate and subsequently rFVIIIFc, citrated whole blood was sampled at various time points and the clot formation following recalcification was monitored by ROTEM. Despite the variable baseline CT due to residue FVIII levels prior to Advate or rFVIIIFc dosing, both products effectively corrected the CT to comparable levels 30 minutes post-injection. In addition, the improvement in CT was better sustained at and after 3 hours post-injection of 25 IU/kg of rFVIIIFc relative to Advate in the subject dosed at this low dose. However, the differential improvement of rFVIIIFc versus Advate was much less appreciable at the 65 IU/kg dose.

Tables

TABLE 1

Polynucleotide Sequences

A. B-Domain Deleted FVIIIFc

(i) B-Domain Deleted FVIIIFc Chain DNA Sequence (FVIII signal peptide underlined, Fc region in bold) (SEQ ID NO: 1, which encodes SEQ ID NO: 2)

```
  661                                A TGCAAATAGA GCTCTCCACC TGCTTCTTTC
  721 TGTGCCTTTT GCGATTCTGC TTTAGTGCCA CCAGAAGATA CTACCTGGGT CCAGTGGAAC
  781 TGTCATGGGA CTATATGCAA AGTGATCTCG GTGAGCTGCC TGTCCACGCA AGATTTCCTC
  841 CTAGAGTGCC AAAATCTTTT CCATTCAACA CCTCAGTCGT GTACAAAAAG ACTCTGTTTG
  901 TAGAATTCAC GGATCACCTT TTCAACATCG CTAAGCCAAG GCCACCCTGG ATGGGTCTGC
  961 TAGGTCCTAC CATCCAGGCT GAGGTTTATG ATACAGTGGT CATTACACTT AAGAACATGG
 1021 CTTCCCATCC TGTCAGTCTT CATGCTGTTG GTGTATCCTA CTGGAAAGCT TCTGAGGGAG
 1081 CTGAATATGA TGATCAGACC AGTCAAAGGG AGAAAGAAGA TGATAAAGTC TTCCCTGGTG
 1141 GAAGCCATAC ATATGTCTGG CAGGTCCTGA AAGAGAATGG TCCAATGGCC TCTGACCCAC
 1201 TGTGCCTTAC CTACTCATAT CTTTCTCATG TGGACCTGGT AAAAGACTTG AATTCAGGCC
 1261 TCATTGGAGC CCTACTAGTA TGTACACAAG GGAGTCTGGC CAAGGAAAAG ACACAGACCT
 1321 TGCACAAATT TATACTACTT TTTGCTGTAT TTGATGAAGG GAAAAGTTGG CACTCAGAAA
 1381 CAAAGAACTC CTTCATGCAG GATAGGGATG CTGCATCTGC TCGGGCCTGG CCTAAAATGC
 1441 ACACAGTCAA TGGTTATGTA AACAGGTCTC TGCCAGGTCT GATTGGATGC CACAGGAAAT
 1501 CAGTCTATTG GCATGTGATT GGAATGGGCA CCACTCCTGA AGTGCACTCA ATATTCCTCG
 1561 AAGGTCACAC ATTTCTTGTG AGGAACCATC GCCAGGCGTC CTTGGAAATC TCGCCAATAA
 1621 CTTTCCTTAC TGCTCAAACA CTCTTGATGG ACCTTGGACA GTTTCTACTG TTTTGTCATA
 1681 TCTCTTCCCA CCAACATGAT GGCATGGAAG CTTATGTCAA ACTAGACAGC TGTCCAGAGG
```

TABLE 1-continued

Polynucleotide Sequences

1741 AACCCCAACT ACGAATCAAA AATAATGAAG AAGCGGAAGA CTATGATGAT GATCTTACTG
1801 ATTCTGAAAT GGATGTGGTC AGGTTTGATG ATGACAACTC TCCTTCCTTT ATCCAAATTC
1861 GCTCAGTTGC CAAGAAGCAT CCTAAAACTT GGGTACATTA CATTGCTGCT GAAGAGGAGG
1921 ACTGGGACTA TGCTCCCTTA GTCCTCGCCC CCGATGACAG AAGTTATAAA AGTCAATATT
1981 TGAACAATGG CCCTCAGCGG ATTGGTAGGA AGTACAAAAA AGTCCGATTT ATGGCATACA
2041 CAGATGAAAC CTTTAAGACT CGTGAAGCTA TTCAGCATGA ATCAGGAATC TTGGGACCTT
2101 TACTTTATGG GGAAGTTGGA GACACACTGT TGATTATATT TAAGAATCAA GCAAGCAGAC
2161 CATATAACAT CTACCCTCAC CGAATCACTG ATGTCCGTCC TTTGTATTCA AGGAGATTAC
2221 CAAAACGTGT AAAACATTTG AAGGATTTTC CAATTCTGCC AGGAGAAATA TTCAAATATA
2281 AATGGACAGT GACTGTAGAA GATGGGCCAA CTAAATCAGA TCCTCGGTGC CTGACCCGCT
2341 ATTACTCTAG TTTCGTTAAT ATGGAGAGAG ATCTAGCTTC AGGACTCATT GGCCCTCTCC
2401 TCATCTGCTA CAAAGAATCT GTAGATCAAA GAGGAAACCA GATAATGTCA GACAAGAGGA
2461 ATGTCATCCT GTTTTCTGTA TTTGATGAGA ACCGAAGCTG GTACCTCACA GAGAATATAC
2521 AACGCTTTCT CCCCAATCCA GCTGGAGTGC ACCTTGAGGA TCCAGAGTTC CAAGCCTCCA
2581 ACATCATGCA CAGCATCAAT GGCTATGTTT TTGATAGTTT GCAGTTGTCA GTTTGTTTGC
2641 ATGACGTGGC ATACTGGTAC ATTCTAAGCA TTGGAGCACA GACTGACTTC CTTTCTGTCT
2701 TCTTCTCTGG ATATACCTTC ALACACAAAA TGGTCTATGA AGACACACTC ACCCTATTCC
2761 CATTCTCAGG AGAAACTGTC TTCATGTCGA TGGAAAACCC AGGTCTATGG ATTCTGGGGT
2821 GCCACAACTC AGACTTTCGG AACAGAGGCA TGACCGCCTT ACTGAAGGTT TCTAGTTGTG
2881 ACAAGAACAC TGGTGATTAT TACGAGGACA GTTATGAAGA TATTTCACCA TACTTGCTGA
2941 GTAAAAACAA TGCCATTGAA CCAAGAAGCT TCTCTCAAAA CCCACCAGTC TTGAAACGCC
3001 ATCAACGGGA AATAACTCGT ACTACTCTTC ACTCAGATCA AGAGGAAATT GACTATGATG
3061 ATACCATATC AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC
3121 AGAGCCCCCG CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC
3181 TCTGGGATTA TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA
3241 GTGTCCCTCA GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC
3301 CCTTATACCG TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG
3361 AAGTTGAAGA TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT
3421 ATTCTAGCCT TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT
3481 TTGTCAAGCC TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA
3541 CTAAAGATGA GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG
3601 ATGTGCACTC AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCTG
3661 CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA
3721 CCAAAAGCTG GTACTTCACT GAAAATATGG AAAGAAACTG CAGGCCTCCC TGCAATATCC
3781 AGATGCAAGA TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA
3841 TGGATACACT ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA
3901 GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC
3961 GAAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG
4021 TGGAAATGTT ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC
4081 TACATGCTGG GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG
4141 GAATGGCTTC TGGATACACT AGAGATTTTC AGATTACAGC TTCAGCACAA TATGGACAGT
4201 GCGCCCCAAA GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG
4261 AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA
4321 CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA
4381 GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT
4441 TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG
4501 CTCGATACAT CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT
4561 TGATGGGCTG TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT
4621 CACATGCACA GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT
4681 CAAAAGCTCG ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC
4741 CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC
4801 AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC
4861 AAGATGGCCA TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA
4921 ATCAAGACTC CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC
4981 TTCGAATTCA CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGCGCT
5041 GCGAGGCACA GGACCTCTAC **GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCAGAAC**
5101 **TCCTGGGCGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT**
5161 **CCCGGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA**
5221 **AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG**
5281 **AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC**
5341 **TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA**
5401 **AAACCATCTC CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT**
5461 **CCCGGGATGA GCTGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC**
5521 **CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA**
5581 **CGCCTCCCGT GTTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA**
5641 **AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA**
5701 **ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA A**

(ii) Fc DNA sequence (mouse Igκ signal peptide underlined) (SEQ ID NO: 3, which encodes SEQ ID NO: 4)

7981 ATGGA GACAGACACA
8041 CTCCTGCTAT GGGTACTGCT GCTCTGGGTT CCAGGTTCCA CTGGTGACAA AACTCACACA
0101 TGCCCACCGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA
8161 AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
8221 GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
8281 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
8341 CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC
8401 AAAGCCCTCC CTGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA

TABLE 1-continued

Polynucleotide Sequences

```
 8461 CCACAGGTGT ACACCCTGCC CCCATCCCGC GATGAGCTGA CCAAGAACCA GGTCAGCCTG
 8521 ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
 8581 CAGCCGGAGA ACAACTACAA GACCACGCCT CCCCTGTTGG ACTCCGACGG CTCCTTCTTC
 8641 CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCCCATGC
 8701 TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
 8761 GGTAAA

B. Full Length FVIIIFc
(i) Full Length FVIIIFc DNA Sequence (FVIII signal peptide underlined,
Fc region in bold) (SEQ ID NO: 5, which encodes SEQ ID NO: 6)
  661                                                ATG CAAATAGAGC TCTCCACCTG
  721 CTTCTTTCTG TGCCTTTTGC GATTCTGCTT TAGTGCCACC AGAAGATACT ACCTGGGTGC
  781 AGTGGAACTG TCATGGGACT ATATGCAAAG TGATCTCGGT GAGCTGCCTG TGGACGCAAG
  841 ATTTCCTCCT AGAGTGCCAA AATCTTTTCC ATTCAACACC TCAGTCGTCT ACAAAAAGAC
  901 TCTGTTTGTA GAATTCACGG ATCACCTTTT CAACATCGCT AAGCCAAGGC CACCCTGGAT
  961 GGGTCTGCTA GGTCCTACCA TCCAGGCTGA GGTTTATGAT ACAGTGGTCA TTACACTTAA
 1021 GAACATGGCT TCCCATCCTG TCAGTCTTCA TGCTGTTGGT GTATCCTACT GGAAAGCTTC
 1081 TGAGGGAGCT GAATATGATG ATCAGACCAG TCAAAGGGAG AAAGAAGATG AAACTGTCTT
 1141 CCCTGGTGGA AGCCATACAT ATGTCTGGCA GGTCCTGAAA GAGAATGGTC CAATGGCCTC
 1201 TGACCCACTG TGCCTTACCT ACTCATATCT TTCTCATGTG GACCTGGTAA AAGACTTGAA
 1261 TTCAGGCCTC ATTGGAGCCC TACTAGTATG TAGAGAAGGG AGTCTGGCCA AGCAAAAGAC
 1321 ACAGACCTTG CACAATTCCA TACTACTTTT TGCTGTATTT GATGAAGGGA ATGTCTGGCA
 1381 CTCAGAAACA AAGAACTCCT TGATGCAGGA CAGGGATGCT GCATCTGCTC GGGCCTGGCC
 1441 TAAAATGCAC ACAGTCAATG GTTATGTAAA CAGGTCTCTG CCAGGTCTGA TTGGATGCCA
 1501 CAGGAAATCA GTCTATTGGC ATGTGATTGG AATGGGCACC ACTCCTGAAG TGCACTCAAT
 1561 ATTCCTCGAA GGTCACACAT TTCTTGTGAG GAACCATCGC CAGGCGTCCT TGGAAATCTC
 1621 GCCAATAACT TTCCTTACTG CTCAAACACT CTTGATGGAC CTTGGACAGT TTCTACTGTT
 1681 TTGTCATATC TCTTCCCACC AACATGATGG CATGGAAGCT TATGTCAAAG TAGACAGCTC
 1741 TCCAGAGGAA CCCCAACTAC GAATGAAAAA TAATCAAGAA GCGGAAGACT ATGATGATGA
 1801 TCTTACTGAT TCTGAAATGG ATGTGGTCAG GTTTGATGAT GACAACTCTC CTTCCTTTAT
 1861 CCAAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACTTGG GTACATTACA TTGCTGCTGA
 1921 AGAGGAGGAC TGGGACTATG CTCCCTTAGT CCTCGCCCCC GATGACAGAA GTTATAAAAG
 1981 TCAATATTTG AACAATGGCC CTCAGCGGAT TGGTAGGAAG TACAAAAAAG TCCGATTTAT
 2041 GGCATACACA GATGAAACCT TTAAGACTCG TGAAGCTATT CAGCATGAAT CAGGAATCTT
 2101 GGGACCTTTA CTTTATGGGG AAGTTGGAGA CACACTGTTG ATTATATTTA AGAATCAAGC
 2161 AAGCAGACCA TATAACATCT ACCCTCACGG AATCACTGAT CTCCGTCCTT TGTATTCAAG
 2221 CAGAGTTCCA AAAGGTCTAA AACATTTGAA GGATTTTCCA ATTCTGCCAG GAGAAATATT
 2281 CAAATATAAA TGGACAGTGA CTGTAGAAGA TGGGCCAACT AAATCAGATC CTCGGTGCCT
 2341 GACCCGCTAT TACTCTAGTT TCGTTAATAT GGAGAGAGAT CTAGCTTCAG GACTCATTGG
 2401 CCCTCTCCTC ATCTGCTACA AAGAATCTGT AGATCAAAGA GGAAACCAGA TAATGTCAGA
 2461 CAAGAGGAAT GTCATCCTGT TTTCTGTATT TGATGAGAAC CGAAGCTGGT ACCTCACAGA
 2521 GAATATACAA CGCTTTCTCC CCAATCCAGC TGGAGTGCAG CTTGAGGATC CAGAGTTCCA
 2581 AGCCTCCAAC ATCATGCACA GCATCAATGG CTATCTTTTT GATAGTTTGC AGTTGTCAGT
 2641 TTGTTTGCAT GAGGTGGCAT ACTGGTACAT TCTAAGCATT GGAGCACAGA CTGACTTCCT
 2701 TTCTGTCTTC TTCTCTGGAT ATACCTTCAA ACACAAAATG GTCTATGAAG ACACACTCAC
 2761 CCTATTCCCA TTCTCAGGAG AAACTGTCTT CATGTCGATG GAAAACCCAG GTCTATGGAT
 2821 TCTGGGGTGC CACAACTCAG ACTTTCGGAA CAGAGGCATG ACCGCCTTAC TGAAGGTTTC
 2881 TAGTTGTGAC AAGAACACTG GTGATTATTA CGAGGACAGT TATGAAGATA TTTCAGCATA
 2941 CTTGCTGAGT AAAAACAATG CCATTGAACC AAGAAGCTTC TCCCAGAATT CAAGACACCC
 3001 TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA GAAAATGACA TAGAGAAGAC
 3061 TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA CAAAATGTCT CCTCTAGTGA
 3121 TTTGTTGATG CTCTTGCGAC AGAGTCCTAC TCCACATGGG CTATCCTTAT CTGATCTCCA
 3181 AGAAGCCAAA TATGAGACTT TTTCTGATGA TCCATCACCT GGAGCAATAG ACAGTAATAA
 3241 CAGCCTGTCT GAAATGACAC ACTTCAGGCC ACAGCTCCAT CACAGTGGGG ACATGGTATT
 3301 TACCCCTGAG TCAGGCCTCC AATTAAGATT AAATGAGAAA CTGGGGACAA CTGCAGCAAC
 3361 AGAGTTGAAG AAACTGTCTT TCAAAGTTTC TAGTACATCA AATAATCTGA TTTCAACAAT
 3421 TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT TCCTTAGGAC CCCCAAGTAT
 3481 GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT GGCAAAAAGT CATCTCCCCT
 3541 TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT AATGATTCAA AGTTGTTAGA
 3601 ATCAGGTTTA ATGAATAGCC AAGAAAGTTC ATGGGGAAAA AATGTATCGT CAACAGAGAG
 3661 TGGTAGGTTA TTTAAAGGGA AAAGAGCTCA TGGACCTGCT TTGTTGACTA AAGATAATGC
 3721 CTTATTCAAA GTTAGCATCT CTTTGTTAAA GACAAACAAA ACTCCCACTA ATTCAGCAAC
 3781 TAATAGAAAG ACTCACATTG ATGGCCCATC ATTATTAATT GAGAATAGTC CATCAGTCTG
 3841 GCAAAATATA TTAGAAAGTG ACACTGAGTT TAAAAAACTC ACACCTTTGA TTCTTGTCTG
 3901 AATGCTTATG CACAAAAATG CTACAGCTTT GAGGCTAAAT CATATGTCAA ATAAAACTAC
 3961 TTCATCAAAA AACATGGAAA TGGTCCAACA GAAAAAAGAG GGCCCCATTC CACCAGATGC
 4021 ACAAAATCCA GATATGTCGT TCTTTAAGAT GCTATTCTTG CCAGAATCAG CAAGGTGGAT
 4081 ACAAAGGACT CATGGAAAGA ACTCTCTGAA CTCTGGGCAA GGCCCCAGTC CAAAGCAATT
 4141 AGTATCCTTA GGACCAGAAA AATCTGTGGA AGGTCAGAAT TTCTTGTCTG AGAAAAACAA
 4201 AGTGGTAGTA GGAAAGGGTG AATTTACAAA GGCGGGTGGA CTCAAAGAGA TGGTTTTTCC
 4261 AAGGAGGAGA AACCTATTTC TTACTAACTT GGATAATTTA CAGATACATA ATACACACAA
 4321 TCAAGAAAAA AAAATTCAGG AAGAAATAGA AAAGAAGGAA ACATTAATCC AAGAGAATGT
 4381 AGTTTTGCCT CAGATACATA CAGTGACTGG CACTAAGAAT TTCATGAAGA ACCTTTTCTT
 4441 ACTGAGCACT AGGCAAAATG TAGAAGGTTC ATATGACGGG GCATATGCTC CAGTACTTCA
 4501 AGATTTTAGG TCATTAAATG ATTCAACAAA TAGAACAAAG AAACACACAG CTCATTTCTC
 4561 AAAAAAAGGG GAGGAAGAAA ACTTGGAAGG CTTGGGAAAT CAAACCAAGC AAATTGTAGA
 4621 GAAATATGCA TGCACCACAA GGATATCTCC TAATACAAGC CAGCAGAATT TTGTCACGCA
 4681 ACGTAGTAAG AGAGCTTTGA AACAATTCAG ACTCCCACTA GAAGAAACAG AACTTGAAAA
```

TABLE 1-continued

Polynucleotide Sequences

```
4741 AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA AACATGAAAC ATTTGACCCC
4801 GAGCACCCTC ACACAGTTAG ACTACAATGA GAAGGAGAAA GGGGCCATTA CTCAGTCTCC
4861 CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA GCAAATAGAT CTCCATTACC
4921 CATTGCAAAG GTATCATCAT TTCCATCTAT TAGACCTATA TATCTGACCA GGGTCCTATT
4981 CCAAGACAAC TCTTCTCATC TTCCAGCAGC ATCTTATAGA AAGAAAGATT CTGGGGTCCA
5041 AGAAAGCAGT CATTTCTTAC AAGGAGCCAA AAAAAATAAC CTTTCTTTAG CATTTCTTAC
5101 CTTGGAGATG ACTGGTGATC AAAGAGAGGT TGGCTCCCTG GGGAGCCTTC CCACAAATTC
5161 AGTCACATAC AACAAACTTC AGAACACTGT TCTCCCGAAA CCAGACTTGC CCAAAACATC
5221 TGGCAAAGTT GAATTCCTTC CAAAAGTTCA CATTTATCAG AAGGACCTAT TCCCTACGGA
5281 AACTAGCAAT GGGTCTCCTG GCCATCTGGA TCTCGTGGAA GGGAGCCTTC TTCAGGGAAC
5341 AGAGGGAGCG ATTAAGTGGA ATGAAGCAAA CAGACCTGGA AAAGTTCCCT TTCTGAGAGT
5401 AGCAACAGAA AGCTCTGCAA AGACTCCCTC CAAGCTATTG GATCCTCTTG CTTGGGATAA
5461 CCACTATGGT ACTCAGATAC CAAAAGAAGA GATGAAAATC CAAGAGAAGT CACCAGAAAA
5521 AACAGCTTTT AAGAAAAAGG ATACCATTTT GTCCCTGAAC GCTTGTGAAA GCAATCATGC
5581 AATAGCAGCA ATAAATGAGG CAAAAGAAGA GCCCGAAATA GAAGTCACCT GGGCAAAGCA
5641 AGGTAGGACT GAAAGGCTGT GCTCTCAAAA CCCACCAGTC TTGAAACGCC ATCAACGGGA
5701 AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATG ATACCATATC
5761 AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC AGAGCCCCCG
5821 CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC TCTGGGATTA
5881 TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA GTGTCCCTCA
5941 GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC CCTTATACCG
6001 TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGACCAG AAGTTGAAGA
6061 TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT ATTCTAGCCT
6121 TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT TTGTCAAGCC
6181 TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA CTAAAGATGA
6241 GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG ATGTGCACTC
6301 AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCTG CTCATGGGAG
6361 ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA CCAAAAGCTG
6421 GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481 TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541 ACCTGGCTTA.GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA GCATGGGCAG
6601 CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC GAAAAAAAGA
6661 GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG TGGAAATGTT
6721 ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC TACATGCTGG
6781 GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG GAATGGCTTC
6841 TGGACACATT AGAGATTTTC AGATTACAGC TTCACCACAA TATGGACAGT GGGCCCCAAA
6901 GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG AGCCCTTTTC
6961 TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA CCCAGGGTGC
7021 CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA GTCTTGATGG
7081 GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT TCTTTGGCAA
7141 TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG CTCGATACAT
7201 CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT TGATGGGCTG
7261 TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT CAGATGCACA
7321 GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT CAAAACCTCG
7381 ACTTCACCTC CAACCCAGGA GTAATCCCTC CACACCTCAC GTGAATAATC CAAAAGAGTG
7441 GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC AGGGAGTAAA
7501 ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561 TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA ATCAAGACTC
7621 CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681 CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741 GGACCTCTAC GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCAGAAC TCCTGGGCGG
7801 ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC
7861 TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG
7921 GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA
7981 CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA
8041 GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC
8101 CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA
8161 GCTGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT
8221 CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT
8281 GTTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG
8341 GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC
8401 GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA A
```

(ii) Fc (same sequence as A (ii) (SEQ ID NO: 3))]

C. (i) Heavy Chain (HC)-Fc DNA sequence (no linker between HC and Fc) (signal peptide underlined, Fc region in bold)(SEQ ID NO: 7, which encodes SEQ ID NO: 8)

```
   1 ATGCAAATAG ACCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
  61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGATCTC
 121 GGTGAGCTGC CTGTGGACGC AAGATTTCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
 181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
 241 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
 361 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGCAAGTG ATGATCAGAC CAGTCAAAGG
 421 GAGAAAGAAG ATGATAAAGT CTTCCCTCGT GGAAGCCATA CATATGTCTG GCCTGACCTG
 481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
 541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
```

TABLE 1-continued

Polynucleotide Sequences

601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGLACCAT
901 GGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AGACAATGAA
1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG
1441 TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT GAAGGATTTT
1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAGTAGACAG TGACTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA TATGGAGAGA
1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAACAATC TCTAGATCAA
1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
1861 CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
1921 TTTGATAGTT TGCAGTTGTC AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101 ATGGAAAACC CAGGTCTATG GATTCTGGCG TGCCACAACT CAGACTTTCG GAACAGAGGC
2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGA**GAC**
2281 **AAAACTCACA CATGCCCACC GTGCCCAGCT CCAGAACTCC TGGGCGGACC GTCAGTCTTC**
2341 **CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC**
2401 **GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC**
2461 **GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT**
2521 **GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA AMGGCAAGGA GTACAAGTGC**
2581 **AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG**
2641 **CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGATGAGCT GACCAAGAAC**
2701 **CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG**
2761 **GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGTT GGACTCCGAC**
2821 **GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC**
2881 **GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC**
2941 **TCCCTGTCTC CGGGTAAA**

C. (ii)
Heavy Chain (HC)-Fc DNA sequence (5 amino acid linker between HC and Fc) (signal peptide underlined, Fc region in bold, 5 amino acid linker is double-underlined) (SEQ ID NO: 9, which encodes SEQ ID NO: 10)

1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGCACTC
121 GGTGAGCTGC CTGTGGACGC AAGATTTCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
241 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
361 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCTG
481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
601 GGGAGTCTGG CCACAGGAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT CCTTGATGCA GGATAGGGAT
721 CCTGCATCTG CTCGCGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
901 CGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCACTTG CCAAGAAGCA TCCTAAAACT
1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG
1441 TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT GAAGGATTTT
1561 CCAATTCTGC CAGCAGAAAT ATTCAAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA TATGGAGAGA
1681 GATATACCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA
1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
1861 CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
1921 TTTGATAGTT TGCAGTTGTC AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC

TABLE 1-continued

Polynucleotide Sequences

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 1981 ATTGCACCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
 2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
 2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG GAACAGAGGC
 2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
 2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAAGC
 2281 TTCTCCCAGA ATGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC
 2341 GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC
 2401 CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
 2461 TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
 2521 AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC
 2581 AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC
 2641 TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT
 2701 GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
 2761 ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
 2821 GTGTTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG
 2881 TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
 2941 ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAA
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C. (iii) Light Chain (LC)-Fc DNA sequence (signal peptide underlined, Fc region in bold) (SEQ ID NO: 11, which encodes SEQ ID NO: 12)

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    1 ATGGAGACAG ACACACTCCT GCTATGGGTA CTGCTGCTCT GGGTTCCAGG TTCCACTGGT
   61 GAAATAACTC GTACTACTCT TCAGTCAGAT CAAGAGGAAA TTGACTATGA TATTACTCTA
  121 TCAGTTGAAA TGAAGAAGGA AGATTTTGAC ATTTATGATG AGGATGAAAA TCAGAGCCCC
  181 CGCAGCTTTC AAAAGAAAAC ACGACACTAT TTTATTGCTG CAGTGGAGAG GCTCTGGGAT
  241 TATGGGATGA GTAGCTCCCC ACATGTTCTA AGAAACAGGG CTCAGAGTGG CAGTGTCCCT
  301 CAGCTCCAGA AAGTTGTTTT CCAGGAATTT ACTGATGGCT CCTTTACTCA GCCCTTATAC
  361 CGTGGAGAAC TAAATGAACA TTTGGGACTC CTGGGGCCAT ATATAAGAGC AGAAGTTGAA
  421 GATAATATCA TGGTAACTTT CAGAAATCAG GCCTCTCGTC CCTATTCCTT CTATTCTAGC
  481 CTTATTTCTT ACGCAGAAGA TCAGAGGCAA GGAGCAGTAC CTAGAAAAAA CTTTGTCAAG
  541 CCTAATGAAA CCAAAACTTA CTTTTGGAAA GTGCAACATC ATATGGCACC CACTAAAGAT
  601 GAGTTTGACT GCAAAGCCTG GGCTTATTTC TCTGATGTTG ACCTGGAAAA AGATGTGCAC
  661 TCAGGCCTGA TTGGACCCCT TCTGGTCTGC CACACTAACA CACTGAACCC TGCTCATGGG
  721 AGACAAGTGA CAGTACAGGA ATTTGCTCTG TTTTTCACCA TCTTTGATGA GACCAAAAGC
  781 TGGTACTTCA CTCAAAATAT GGAAACAAAC TGCAGGGCTC CCTGCAATAT CCAGATGGAA
  841 GATCCCACTT TTAAAGAGAA TTATCGCTTC CATGCAATCA ATGGCTACAT AATGGATACA
  901 CTACCTGGCT TAGTAATGGC TCAGGATCAA AGGATTCGAT GGTATCTGCT CAGCATGGGC
  961 AGCAATGAAA ACATCCATTC TATTCATTTC AGTGGACATG TGTTCACTGT ACGAAAAAAA
 1021 GAGGAGTATA AAATGGCACT GTACAATCTC TATCCAGGTG TTTTTGAGAC AGTGGAAATG
 1081 TTACCATCCA AAGCTGGAAT TTGGCGGGTG GAATGCCTTA TTGGCGAGCA TCTACATGCT
 1141 GGGATGAGCA CACTTTTTCT GGTGTACAGC RATRAGTGTC AGACTCCCCT GGGAATGGCT
 1201 TCTGGACACA TTAGAGATTT TCAGATTACA GCTTCAGGAC AATATGGACA GTGGGCCCCA
 1261 AAGCTGGCCA GACTTCATTA TTCCGGATCA ATCAATGCCT GGAGCACCAA GGAGCCCTTT
 1321 TCTTGGATCA AGGTGGATCT GTTGGCACCA ATGATTATTC ACGGCATCAA GACCCAGGGT
 1381 GCCCGTCAGA AGTTCTCCAG CCTCTACATC TCTCAGTTTA TCATCATGTA TAGTCTTGAT
 1441 GGGAAGAAGT GGCAGACTTA TCGAGGAAAT TCCACTGGAA CCTTAATGGT CTTCTTTGGC
 1501 AATGTGGATT CATCTGGGAT AAAACACAAT ATTTTTAACC CTCCAATTAT TGCTCGATAC
 1561 ATCCGTTTGC ACCCAACTCA TTATAGCATT CGCAGCACTC TTCGCATGGA GTTGATGGGC
 1621 TGTGATTTAA ATAGTTGCAG CATGCCATTG GGAATGGAGA GTAAAGCAAT ATCAGATGCA
 1681 CAGATTACTG CTTCATCCTA CTTTACCAAT ATGTTTGCCA CCTGGTCTCC TTCAAAAGCT
 1741 CGACTTCACC TCCAAGGGAG GAGTAATGCC TGGAGACCTC AGGTGAATAA TCCAAAAGAG
 1801 TGGCAGCAGG TGGACTTCCA GAAGACAATG AAAGTCACAG GAGTAACTAC TCAGGGAGTA
 1861 AAATCTCTGC TTACCAGCAT GTATGTGAAG GAGTTCCTCA TCTCCAGCAG TCAAGATGGC
 1921 CATCAGTGGA CTCTCTTTTT TCAGAATGGC AAAGTAAAGG TTTTTCAGGG AAATCAAGAC
 1981 TCCTTCACAC CTGTGGTGAA CTCTCTAGAC CCACCGTGCC TGACTCGCTA CCTTCGAATT
 2041 CACCCCCAGA GTTGGGTGCA CCAGATTGCC CTGAGGATGG AGGTTCTGGG CTGCGAGGCA
 2101 CAGGACCTCT ACGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC
 2161 GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC
 2221 CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
 2281 TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
 2341 AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC
 2401 AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC
 2461 TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT
 2521 GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
 2581 ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
 2641 GTGTTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG
 2701 TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
 2761 ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAA
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TABLE 2

Polypeptide Sequences

A. B-Domain Deleted FVIII-Fc Monomer Hybrid (BDD FVIIIFc monomer dimer): created by coexpressing BDD FVIIIFc and Fc chains.
Construct = HC-LC-Fc fusion. An Fc expression cassette is cotransfected with BDDFVIII-Fc to

TABLE 2-continued

Polypeptide Sequences

generate the BDD FVIIIFc monomer-. For the BDD FVIIIFc chain, the Fc sequence is shown in bold; HC sequence is shown in double underline; remaining B domain sequence is shown in italics. Signal peptides are underlined.

i) B domain deleted FVIII-Fc chain (19 amino acid signal sequence underlined)

(SEQ ID NO: 2)

MQIELSTCFFLCLLRFCFSATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMAS
DPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHT
VNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQH
DGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLA
PDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRP
LYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSD
KRNVILFSVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLSVFF
SGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPR
EPR*SFSQNSQNPPVLKRHQR*EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSP
HVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQPAEP
RKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSW
YFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALY
NLYPGVFETVMELPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYGQWAPKLARLHYSGSINAWS
TKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGQVNNPKEWLQVDFQKT
MKVIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNA
WRPTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVL
GCEAQDLY**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**

ii) Fc chain (20 amino acid heterologous signal peptide from mouse Igκ chain underlined)

(SEQ ID NO: 4)

METDTLLLWVLLLWVPGSTGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNFVSCSVMHEALHNHYTQKSLSLSPGK

B. Full length FVIIIFc monomer hybrid (Full length FVIIIFc monomer dimer):
created by coexpressing FVIIIFc and Fc chains.
Construct = HC-B-LC-Fc fusion. An Fc expression cassette is cotransfected with full length FVIII-Fc to generate the full length FVIIIFc monomer. For the FVIIIFc chain, the Fc sequence is shown in bold; HC sequence is shown in double underline; B domain sequence is shown in italics. Signal peptides are underlined.

i) Full length FVIIIFc chain (FVIII signal peptide underlined

(SEQ ID NO: 6)

MQIELSTCFFLCLLRFCFSATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPRPP
WMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASDPLC
LTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVN
RSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQY
LNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKH
LKDFPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENR
SWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMVYEDTLT
LFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPR*SFSQNSRHPSTRQ
KQFNATTIPENDIEKTDPWFAHRTPMPKIQNVSSSDLLMLLRQSPTPHGLSLSDLQEAKYETFSDDPSPGAIDSNNSLSEMTHFRPQ
LHHSGDMVFTPESGLQLRLNEKLGTTAATELKKLDFKVSSTSNNLISTIPSDNLAAGTDNTSSLGPPSMPVHYDSQLDTTLFGKKSS
PLTESGGPLSLSEENNDSKLLESGLMNSQESSWGKNVSSTESGRLFKGKRAHGPALLTKDNALFKVSISLLKTNKTSNNSATNRKTH
IDGPSLLIENSPSVWQNILESDTEFKKVTPLIHDRMLMDKNATALRLNHMSNKTTSSKNMEMVQQKKEGPIPPDAQNPDMSFFKMLF
LPESARWIQRTHGKNSLNSGQGPSPKQLVSLGPEKSVEGQNFLSEKNKVVVGKGEFTKDVGLKEMVFPSSRNLFLTNLDNLHENNTH
NQEKKIQEEIEKKETLIQENVVLPQIHTVTGTKNFMKNLFLLSTRQNVEGSYDGAYAPVLQDFRSLNDSTNRTKKHTAHFSKKGEEE
NLEGLGNQTKQIVEKYACTTRISPNTSQQNFVTQRSKRALKQFRLPLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEK
GAITQSPLSDCLTRSHSIPQANRSPLPIAKVSSFPSIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQESSHFLQGAKKNNLSLAILT
LEMTGDQREVGSLGTSATNSVTYKKVENTVLPKPDLPKTSGKVELLPKVHIYQKDLFPTETSNGSPGHLDLVEGSLLQGTEGAIKWN
EANRPGKVPFLRVATESSAKTPSKLLDPLAWDNHYGTQIPKEEWKSQEKSPEKTAFKKKDTILSLNACESNHAIAAINEGQNKPEIE
VTWAKQGRTERLCSQNPPVLKRHQR*EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYG
MSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQR
QGAEPRKNFVKPNETKTYFWKQVHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFD
ETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSNENISHIHFSGHVFTVRKKEEY
KMALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYGQWAPKLARLHYSGS
INAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPII
ARYIRLJPTNYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQV
DFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIAL
RMEVLGCEAQDLY**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**

ii) Fc chain (20 amino acid heterologous signal peptide from mouse Igκ chain underlined)

(SEQ ID NO: 4)

METDTLLLWVLLLWVPGSTGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

TABLE 2-continued

Polypeptide Sequences

C. FVIII-Fc Heterodimer Hybrid
This is made by cotransfecting HC-Fc and LC-Fc constructs. Two HC-Fc constructs have been made. One has no linker between HC and Fc (HC-Fc) while the other has a 5 amino acid linker between HC and Fc (HC + F-Fc). The FVIII signal peptide was used for the HC-Fc constructs, while the Igκ signal sequence was used for the LC-Fc construct.

(i) HC-Fc (Fc sequence is shown in bold, signal peptide underlined)

(SEQ ID NO: 8)

MQIELSTCFFLCLLRFCFSATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPRPPW
MGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASDPLCLT
YSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSL
PGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSC
PEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGP
QRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPI
LPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSWTLTEN
IQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMVYEDTLTLFPFSGET
VFMSMENGPLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPR**DKTHTCPPCPAPELLGGPSVFLFP**
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
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(ii) HC + 5-Fc (Fc sequence is shown in bold, 5 amino acid linker sequence (from the B domain of FVIII) is shown in italics, signal peptide underlined.)

(SEQ ID NO: 10)

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(iii) LC-Fc6His (Fc sequence is shown in bold, signal peptide underlined.)

(SEQ ID NO: 12)

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TABLE 3

Whole blood clotting time (WBCT) determination in hemophilia A mice after a single intravenous dose of 50 IU/kg rFVIIIFc or ReFacto ®.

A.

Treatment	Animal Number	Time of Blood Collection, hr: Pre-dose	0.25	24	36	42	96	113	120
		WBCT, min							
50 IU/kg ReFacto ®	1	>60	18	>60	ND	ND			
	2	>60	5	16	>60	ND			
	3	>60	4	7	>60	ND			
	4	>60	7	8	10	>60			
	5	>60	6	9	16	>60			
	6	>60	5	15	>60	ND			

TABLE 3-continued

Whole blood clotting time (WBCT) determination in hemophilia A mice after a single intravenous dose of 50 IU/kg rFVIIIFc or ReFacto ®.							
50 IU/kg rFVIIIFc	7	>60	7	8	>60	ND
	8	>60	5	8	>60	ND
	9	>60	4	16	>60	ND
	10	>60	3	11	4	>60
	11	>60	3	9	>60	ND
	12	>60	4	6	>60	ND

B.

		Time of Blood Collection, hr					
Treatment	Animal Number	Pre-dose	0.25	24	48	96	120
		WBCT, min					
50 IU/kg	1	>60	11	15	>60	>60	ND
ReFacto ®	2	>60	3	3	>60	>60	>60
	3	>60	4	6	>60	>60	>60
50 IU/kg	4	>60	3	5	5	>60	>60
rFVIIIFc	5	>60	3	6	7	13	>60
	6	>60	5	8	9	9	>60

ND = Not determined since previous time point was >60 min

TABLE 4

PK Parameters after a single intravenous dose in hemophilia A mice (50 IU/kg)					
Treatment	C_{max} (IU/mL)	AUC (hr · IU/mL)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vss (mL/kg)
rFVIIIFc	1.56	22.6	11.1	2.09	28.4
ReFacto ®®	0.67	6.94	5.0	7.2	43.8
Advate ®	0.47	3.90	7.1	12.8	103

TABLE 5

PK Parameters after a single intravenous dose in hemophilia A dogs (125 IU/kg rFVIIIFc, 114 and 120 IU/kg ReFacto ®)

A. PK determined from chromogenic activity data

Treatment	C_{max} (IU/mL)	AUC (hr · IU/mL	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vz (mL/kg)
rFVIIIFc	2.0 ± 0.54	25.9 ± 6.47	15.4 ± 0.3	5.1 ± 1.4	113 ± 29
ReFacto ®®*	2.0	18.2	7.4	6.5	68.7

B. PK determined from ELISA data

Treatment	C_{max} (ng/mL)	AUC (hr · ng/mL	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vz (mL/kg)
rFVIIIFc	210 ± 33	2481 ± 970	15.7 ± 1.7	6.2 ± 3.0	144 ± 83
ReFacto ®®*	211	1545	6.9	8.7	85

Mean ± sd, n = 4 for rFVIIIFc, n = 2 for ReFacto ®

*sd not reported for ReFacto ® since there were just two dogs

TABLE 6

Clotting activity measured by aPTT in hemophilia A dogs after a single intravenous dose with rFVIIIFc or ReFacto ®.

		aPTT, sec	
Dog ID	Treatment	PreDose	5 min post dose
M10	rFVIIIFc	86.5	53.6
M11	rFVIIIFc	99.8	56.4
M12	rFVIIIFc	119	68.7
	ReFacto ®	108	60.7
M38	rFVIIIFc	115	76.6
	ReFacto ®	118	68.0

TABLE 7

Plasma Concentration of rFVIIIFc or Xyntha in monkeys administered as a single intravenous dose of 125 IU/kg measured by ELISA.

	Group 1			Group 2				
Time, hr	604376	606595	C36195	C36066	C36174	604362	Mean	SD
A. rFVIIIFc concentration in plasma (μg/mL)								
Pre	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ		
0.25	0.400	0.334	0.374	0.348	0.383	0.323	0.360	0.030
4	0.266	0.259	0.236	0.233	0.259	0.217	0.245	0.019
12	0.165	0.152	0.12	0.15	0.161	0.149	0.150	0.016
24	0.079	0.074	0.047	0.08	0.088	0.076	0.074	0.014
36	0.035	0.04	0.022	0.04	0.041	0.046	0.037	0.008
48	0.019	0.021	BLQ	0.021	0.024	0.025	0.022	0.002
B. Xyntha concentration in plasma (μg/mL)								
Pre	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ		
0.25	0.252	0.074	0.155	0.317	0.217	0.167	0.197	0.084
4	0.197	0.159	0.152	0.229	0.19	0.082	0.168	0.051
12	0.137	0.099	0.104	0.166	0.158	0.081	0.124	0.035
24	0.09	0.068	0.051	0.082	0.08	0.084	0.076	0.014
36	0.037	0.043	0.015	0.041	0.035	BLQ	0.034	0.011
48	0.022	BLQ	BLQ	0.017	0.013	BLQ	0.017	0.005

TABLE 8

Plasma Concentration of rFVIIIFc or Xyntha in monkeys administered a single intravenous dose of 125 IU/kg measured by the FVIII-specific chromogenic activity assay (reported in IU/mL).

Time (hr)	Group 1			Group 2		
Predose	604376	606595	C36195	C36066	C36174	604362
A. Xyntha						
0.25	5.62	4.55	5.01	4.5	5.15	3.77
4	3.9	4.05	3.2	3.19	3.46	2.36
12	2.51	2.82	1.69	2.17	2.5	2.01
24	1.67	1.66	1.18	0.95	1.57	1.5
36	0.7	0.85	0.48	0.44	0.85	0.82
48	BLQ	BLQ	BLQ	BLQ	0.38	0.48
B. rFVIIIFc						
0.25	4.31	3.82	3.54	4.13	4.12	3.68
4	3	3.36	2.53	2.7	2.74	2.81
12	2	2.15	1.42	2.28	2.75	2.22
24	1.01	1.17	0.5	1.5	1.61	1.01
36	BLQ	0.52	0.48	0.88	0.72	0.64
48	0.31	BLQ	BLQ	BLQ	BLQ	BLQ
72	BLQ	BLQ	BLQ	BLQ	0.31	BLQ

BLQ = below the limit of quantitation

TABLE 9

PK Parameters of rFVIIIFc after a single 125 IU/kg dose

PK		Group 1			Group 2				
Parameter	units	604376	606595	C36195	C36066	C36174	604362	Average	SD
	rFVIIIFc ELISA Data								
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
Cmax	μg/mL	0.4	0.334	0.374	0.348	0.383	0.323	0.368	0.030
$T_{1/2}$	hr	11.4	13.3	9.3	12.7	12.7	14.1	11.9	1.7
AUC	μg * hr/mL	5.86	5.65	4.37	5.56	4.37	5.58	5.16	0.68
CL	mL/hr/kg	2.15	2.23	2.88	2.27	2.07	2.26	2.32	0.29
Vz	mL/kg	35.3	42.5	38.8	37.9	37.9	46.1	38.5	3.9
MRT	hr	15.3	17	12.1	17.1	17.3	19.2	15.8	2.4
	rFVIIIFc Chromogenic Activity Data								
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
Cmax	IU/mL	4.31	3.82	3.54	4.13	4.12	3.68	3.93	0.30
$T_{1/2}$	hr	13.4	12.0	11.6	17.5	12.4	29.4	16.1	6.9
AUC	IU * hr/mL	74.7	75.5	53.5	92.9	88.9	92.7	79.7	15.2
CL	mL/hr/kg	1.67	1.65	2.34	1.35	1.41	1.35	1.63	0.38
Vz	mL/kg	32.3	28.7	39.2	33.9	25.2	57.2	36.1	11.4
MRT	hr	17.8	16.8	16.9	25	19.2	33.3	21.5	6.5

TABLE 10

PK Parameters of Xyntha after a single IV dose (125 IU/kg)

PK		Group 1			Group 2				
Parameter	units	604376	606595	C36195	C36066	C36174	604362	Average	SD
	Xyntha ELISA Data								
Tmax	hr	0.25	4	0.25	0.25	0.25	0.25	0.88	1.53
Cmax	IU/mL	0.252	0.159	0.155	0.317	0.217	0.167	0.21	0.06
$T_{1/2}$	hr	13.6	19.9	9.7	11	9.2	nd	12.7	4.4
AUC	IU * hr/mL	5.15	4.39	3.17	5.53	4.79	6.32	5.24	0.74
CL	mL/hr/kg	2.21	2.6	3.59	2.06	2.38	nd	2.57	0.61
Vz	mL/kg	43.4	74.7	50.1	32.9	31.5	nd	46.5	17.5
MRT	hr	19	28.4	14	16.1	15.9	nd	18.7	5.7
	Xyntha Chromogenic Activity Data								
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0
Cmax	IU/mL	5.62	4.55	5.01	4.5	5.15	3.77	4.77	0.64
$T_{1/2}$	hr	12.8	14.3	11.4	10.4	11.7	14.6	12.5	1.7
AUC	IU * hr/mL	97.1	104.2	71.3	70.7	94.0	82.8	86.7	14.0
CL	mL/hr/kg	1.29	1.20	1.75	1.77	1.33	1.51	1.48	0.24
Vz	mL/kg	23.7	24.8	28.9	26.6	22.5	31.8	26.4	3.5
MRT	hr	17.8	20.1	16.0	14.8	18.4	23.2	18.4	3.0

TABLE 11

Activation of Factor X

	Km (nM)	Vmax (nM/min)
rFVIIIFc	55.0 ± 5.9	65.6 ± 8.6
BDD FVIII	51.0 ± 8.7	73.5 ± 10.1

TABLE 12

Interaction with Factor IXa

	Kd (nM)	Vmax (nM/min)
rFVIIIFc	2.8 ± 0.4	4.5 ± 0.3
BDD FVIII	2.5 ± 0.3	4.0 ± 1.0

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<210> SEQ ID NO 2
<211> LENGTH: 1684
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: B domain deleted FVIII-Fc chain
<220> FEATURE:
<221> NAME/KEY: SIGNAL
<222> LOCATION: (1)..(19)
<220> FEATURE:
<221> NAME/KEY: mat_peptide
<222> LOCATION: (20)..(1684)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (20)..(753)
<223> OTHER INFORMATION: Heavy chain (HC)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (760)..(773)
<223> OTHER INFORMATION: B domain
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1457)..(1684)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 2

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
-15 -10 -5

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
-1 1 5 10

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
15 20 25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
30 35 40 45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
50 55 60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
65 70 75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser

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        80                  85                  90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
    95                  100                 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110                 115                 120                 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
                130                 135                 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
            145                 150                 155

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
        160                 165                 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
    175                 180                 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190                 195                 200                 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
                210                 215                 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
            225                 230                 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
        240                 245                 250

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
    255                 260                 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270                 275                 280                 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
                290                 295                 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
            305                 310                 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
        320                 325                 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
    335                 340                 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350                 355                 360                 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
                370                 375                 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
            385                 390                 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
        400                 405                 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    415                 420                 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430                 435                 440                 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
                450                 455                 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
            465                 470                 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
        480                 485                 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    495                 500                 505
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Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510                 515                 520                 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
                530                 535                 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
            545                 550                 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
        560                 565                 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
    575                 580                 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590                 595                 600                 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
                610                 615                 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
            625                 630                 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
        640                 645                 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
    655                 660                 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670                 675                 680                 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
                690                 695                 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
            705                 710                 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
        720                 725                 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu
    735                 740                 745

Lys Arg His Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln
750                 755                 760                 765

Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu
                770                 775                 780

Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe
            785                 790                 795

Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp
        800                 805                 810

Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln
    815                 820                 825

Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr
830                 835                 840                 845

Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His
                850                 855                 860

Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile
            865                 870                 875

Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser
        880                 885                 890

Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg
    895                 900                 905

Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val
910                 915                 920                 925
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Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp
               930                 935                 940

Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu
            945                 950                 955

Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His
        960                 965                 970

Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe
    975                 980                 985

Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn  Met Glu Arg Asn Cys
990                 995                 1000                1005

Arg Ala Pro Cys Asn  Ile Gln Met Glu Asp  Pro Thr Phe Lys Glu
                1010                1015                1020

Asn Tyr Arg Phe His  Ala Ile Asn Gly Tyr  Ile Met Asp Thr Leu
                1025                1030                1035

Pro Gly Leu Val Met  Ala Gln Asp Gln Arg  Ile Arg Trp Tyr Leu
                1040                1045                1050

Leu Ser Met Gly Ser  Asn Glu Asn Ile His  Ser Ile His Phe Ser
                1055                1060                1065

Gly His Val Phe Thr  Val Arg Lys Lys Glu  Glu Tyr Lys Met Ala
                1070                1075                1080

Leu Tyr Asn Leu Tyr  Pro Gly Val Phe Glu  Thr Val Glu Met Leu
                1085                1090                1095

Pro Ser Lys Ala Gly  Ile Trp Arg Val Glu  Cys Leu Ile Gly Glu
                1100                1105                1110

His Leu His Ala Gly  Met Ser Thr Leu Phe  Leu Val Tyr Ser Asn
                1115                1120                1125

Lys Cys Gln Thr Pro  Leu Gly Met Ala Ser  Gly His Ile Arg Asp
                1130                1135                1140

Phe Gln Ile Thr Ala  Ser Gly Gln Tyr Gly  Gln Trp Ala Pro Lys
                1145                1150                1155

Leu Ala Arg Leu His  Tyr Ser Gly Ser Ile  Asn Ala Trp Ser Thr
                1160                1165                1170

Lys Glu Pro Phe Ser  Trp Ile Lys Val Asp  Leu Leu Ala Pro Met
                1175                1180                1185

Ile Ile His Gly Ile  Lys Thr Gln Gly Ala  Arg Gln Lys Phe Ser
                1190                1195                1200

Ser Leu Tyr Ile Ser  Gln Phe Ile Ile Met  Tyr Ser Leu Asp Gly
                1205                1210                1215

Lys Lys Trp Gln Thr  Tyr Arg Gly Asn Ser  Thr Gly Thr Leu Met
                1220                1225                1230

Val Phe Phe Gly Asn  Val Asp Ser Ser Gly  Ile Lys His Asn Ile
                1235                1240                1245

Phe Asn Pro Pro Ile  Ile Ala Arg Tyr Ile  Arg Leu His Pro Thr
                1250                1255                1260

His Tyr Ser Ile Arg  Ser Thr Leu Arg Met  Glu Leu Met Gly Cys
                1265                1270                1275

Asp Leu Asn Ser Cys  Ser Met Pro Leu Gly  Met Glu Ser Lys Ala
                1280                1285                1290

Ile Ser Asp Ala Gln  Ile Thr Ala Ser Ser  Tyr Phe Thr Asn Met
                1295                1300                1305

Phe Ala Thr Trp Ser  Pro Ser Lys Ala Arg  Leu His Leu Gln Gly
                1310                1315                1320

Arg Ser Asn Ala Trp  Arg Pro Gln Val Asn  Asn Pro Lys Glu Trp
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                1325                1330                1335

Leu Gln Val Asp Phe  Gln Lys Thr Met Lys  Val Thr Gly Val Thr
                1340                1345                1350

Thr Gln Gly Val Lys  Ser Leu Leu Thr Ser  Met Tyr Val Lys Glu
                1355                1360                1365

Phe Leu Ile Ser Ser  Ser Gln Asp Gly His  Gln Trp Thr Leu Phe
                1370                1375                1380

Phe Gln Asn Gly Lys  Val Lys Val Phe Gln  Gly Asn Gln Asp Ser
                1385                1390                1395

Phe Thr Pro Val Val  Asn Ser Leu Asp Pro  Pro Leu Leu Thr Arg
                1400                1405                1410

Tyr Leu Arg Ile His  Pro Gln Ser Trp Val  His Gln Ile Ala Leu
                1415                1420                1425

Arg Met Glu Val Leu  Gly Cys Glu Ala Gln  Asp Leu Tyr Asp Lys
                1430                1435                1440

Thr His Thr Cys Pro  Pro Cys Pro Ala Pro  Glu Leu Leu Gly Gly
                1445                1450                1455

Pro Ser Val Phe Leu  Phe Pro Pro Lys Pro  Lys Asp Thr Leu Met
                1460                1465                1470

Ile Ser Arg Thr Pro  Glu Val Thr Cys Val  Val Val Asp Val Ser
                1475                1480                1485

His Glu Asp Pro Glu  Val Lys Phe Asn Trp  Tyr Val Asp Gly Val
                1490                1495                1500

Glu Val His Asn Ala  Lys Thr Lys Pro Arg  Glu Glu Gln Tyr Asn
                1505                1510                1515

Ser Thr Tyr Arg Val  Val Ser Val Leu Thr  Val Leu His Gln Asp
                1520                1525                1530

Trp Leu Asn Gly Lys  Glu Tyr Lys Cys Lys  Val Ser Asn Lys Ala
                1535                1540                1545

Leu Pro Ala Pro Ile  Glu Lys Thr Ile Ser  Lys Ala Lys Gly Gln
                1550                1555                1560

Pro Arg Glu Pro Gln  Val Tyr Thr Leu Pro  Pro Ser Arg Asp Glu
                1565                1570                1575

Leu Thr Lys Asn Gln  Val Ser Leu Thr Cys  Leu Val Lys Gly Phe
                1580                1585                1590

Tyr Pro Ser Asp Ile  Ala Val Glu Trp Glu  Ser Asn Gly Gln Pro
                1595                1600                1605

Glu Asn Asn Tyr Lys  Thr Thr Pro Pro Val  Leu Asp Ser Asp Gly
                1610                1615                1620

Ser Phe Phe Leu Tyr  Ser Lys Leu Thr Val  Asp Lys Ser Arg Trp
                1625                1630                1635

Gln Gln Gly Asn Val  Phe Ser Cys Ser Val  Met His Glu Ala Leu
                1640                1645                1650

His Asn His Tyr Thr  Gln Lys Ser Leu Ser  Leu Ser Pro Gly Lys
                1655                1660                1665


<210> SEQ ID NO 3
<211> LENGTH: 741
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Fc region
<220> FEATURE:
<221> NAME/KEY: misc_signal
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: Mouse Ig kappa signal
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<400> SEQUENCE: 3

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gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggagg accgtcagtc 120

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 180

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 240

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 300

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 360

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 420

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgcgatga gctgaccaag 480

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 540

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gttggactcc 600

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 660

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 720

ctctccctgt ctccgggtaa a 741

<210> SEQ ID NO 4
<211> LENGTH: 247
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Fc chain
<220> FEATURE:
<221> NAME/KEY: SIGNAL
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Heterologous signal from Mouse Ig kappa chain
<220> FEATURE:
<221> NAME/KEY: mat_peptide
<222> LOCATION: (21)..(247)

<400> SEQUENCE: 4

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
-20 -15 -10 -5

Gly Ser Thr Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
-1 1 5 10

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
15 20 25

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
30 35 40

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
45 50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65 70 75

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
80 85 90

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
95 100 105

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
110 115 120

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
125 130 135 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145 150 155

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Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            160                 165                 170

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        175                 180                 185

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
    190                 195                 200

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
205                 210                 215                 220

Leu Ser Leu Ser Pro Gly Lys
                225
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<210> SEQ ID NO 5
<211> LENGTH: 7734
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Full length FVIIIFc
<220> FEATURE:
<221> NAME/KEY: misc_signal
<222> LOCATION: (1)..(57)
<223> OTHER INFORMATION: FVIII signal
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7054)..(7734)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 5

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accagaagat actacctggg tgcagtggaa ctgtcatggg actatatgca aagtgatctc    120

ggtgagctgc ctgtggacgc aagatttcct cctagagtgc caaaatcttt tccattcaac    180

acctcagtcg tgtacaaaaa gactctgttt gtagaattca cggatcacct tttcaacatc    240

gctaagccaa ggccaccctg gatgggtctg ctaggtccta ccatccaggc tgaggtttat    300

gatacagtgg tcattacact taagaacatg gcttcccatc ctgtcagtct tcatgctgtt    360

ggtgtatcct actggaaagc ttctgaggga gctgaatatg atgatcagac cagtcaaagg    420

gagaaagaag atgataaagt cttccctggt ggaagccata catatgtctg gcaggtcctg    480

aaagagaatg gtccaatggc ctctgaccca ctgtgcctta cctactcata tctttctcat    540

gtggacctgg taaaagactt gaattcaggc ctcattggag ccctactagt atgtagagaa    600

gggagtctgg ccaaggaaaa gacacagacc ttgcacaaat ttatactact ttttgctgta    660

tttgatgaag ggaaaagttg gcactcagaa acaaagaact ccttgatgca ggatagggat    720

gctgcatctg ctcgggcctg gcctaaaatg cacacagtca atggttatgt aaacaggtct    780

ctgccaggtc tgattggatg ccacaggaaa tcagtctatt ggcatgtgat tggaatgggc    840

accactcctg aagtgcactc aatattcctc gaaggtcaca catttcttgt gaggaaccat    900

cgccaggcgt ccttggaaat ctcgccaata actttcctta ctgctcaaac actcttgatg    960

gaccttggac agtttctact gttttgtcat atctcttccc accaacatga tggcatggaa   1020

gcttatgtca aagtagacag ctgtccagag gaaccccaac tacgaatgaa aaataatgaa   1080

gaagcggaag actatgatga tgatcttact gattctgaaa tggatgtggt caggtttgat   1140

gatgacaact ctccttcctt tatccaaatt cgctcagttg ccaagaagca tcctaaaact   1200

tgggtacatt acattgctgc tgaagaggag gactgggact atgctccctt agtcctcgcc   1260

cccgatgaca gaagttataa aagtcaatat ttgaacaatg gccctcagcg gattggtagg   1320

aagtacaaaa aagtccgatt tatggcatac acagatgaaa cctttaagac tcgtgaagct   1380
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attcagcatg aatcaggaat cttgggacct ttactttatg gggaagttgg agacacactg 1440

ttgattatat ttaagaatca agcaagcaga ccatataaca tctaccctca cggaatcact 1500

gatgtccgtc ctttgtattc aaggagatta ccaaaaggtg taaaacattt gaaggatttt 1560

ccaattctgc caggagaaat attcaaatat aaatggacag tgactgtaga agatgggcca 1620

actaaatcag atcctcggtg cctgacccgc tattactcta gtttcgttaa tatggagaga 1680

gatctagctt caggactcat tggccctctc ctcatctgct acaaagaatc tgtagatcaa 1740

agaggaaacc agataatgtc agacaagagg aatgtcatcc tgttttctgt atttgatgag 1800

aaccgaagct ggtacctcac agagaatata caacgctttc tccccaatcc agctggagtg 1860

cagcttgagg atccagagtt ccaagcctcc aacatcatgc acagcatcaa tggctatgtt 1920

tttgatagtt tgcagttgtc agtttgtttg catgaggtgg catactggta cattctaagc 1980

attggagcac agactgactt cctttctgtc ttcttctctg gatatacctt caaacacaaa 2040

atggtctatg aagacacact caccctattc ccattctcag gagaaactgt cttcatgtcg 2100

atggaaaacc caggtctatg gattctgggg tgccacaact cagactttcg gaacagaggc 2160

atgaccgcct tactgaaggt ttctagttgt gacaagaaca ctggtgatta ttacgaggac 2220

agttatgaag atatttcagc atacttgctg agtaaaaaca atgccattga accaagaagc 2280

ttctcccaga attcaagaca ccctagcact aggcaaaagc aatttaatgc caccacaatt 2340

ccagaaaatg acatagagaa gactgaccct tggtttgcac acagaacacc tatgcctaaa 2400

atacaaaatg tctcctctag tgatttgttg atgctcttgc gacagagtcc tactccacat 2460

gggctatcct tatctgatct ccaagaagcc aaatatgaga ctttttctga tgatccatca 2520

cctggagcaa tagacagtaa taacagcctg tctgaaatga cacacttcag gccacagctc 2580

catcacagtg gggacatggt atttacccct gagtcaggcc tccaattaag attaaatgag 2640

aaactgggga caactgcagc aacagagttg aagaaacttg atttcaaagt ttctagtaca 2700

tcaaataatc tgatttcaac aattccatca gacaatttgg cagcaggtac tgataataca 2760

agttccttag gacccccaag tatgccagtt cattatgata gtcaattaga taccactcta 2820

tttggcaaaa agtcatctcc ccttactgag tctggtggac ctctgagctt gagtgaagaa 2880

aataatgatt caaagttgtt agaatcaggt ttaatgaata gccaagaaag ttcatgggga 2940

aaaaatgtat cgtcaacaga gagtggtagg ttatttaaag ggaaaagagc tcatggacct 3000

gctttgttga ctaaagataa tgccttattc aaagttagca tctctttgtt aaagacaaac 3060

aaaacttcca ataattcagc aactaataga aagactcaca ttgatggccc atcattatta 3120

attgagaata gtccatcagt ctggcaaaat atattagaaa gtgacactga gtttaaaaaa 3180

gtgacacctt tgattcatga cagaatgctt atggacaaaa atgctacagc tttgaggcta 3240

aatcatatgt caaataaaac tacttcatca aaaaacatgg aaatggtcca acagaaaaaa 3300

gagggcccca ttccaccaga tgcacaaaat ccagatatgt cgttctttaa gatgctattc 3360

ttgccagaat cagcaaggtg gatacaaagg actcatggaa agaactctct gaactctggg 3420

caaggcccca gtccaaagca attagtatcc ttaggaccag aaaaatctgt ggaaggtcag 3480

aatttcttgt ctgagaaaaa caaagtggta gtaggaaagg gtgaatttac aaaggacgta 3540

ggactcaaag agatggtttt tccaagcagc agaaacctat ttcttactaa cttggataat 3600

ttacatgaaa ataatacaca caatcaagaa aaaaaaattc aggaagaaat agaaaagaag 3660

gaaacattaa tccaagagaa tgtagttttg cctcagatac atacagtgac tggcactaag 3720

aatttcatga agaacctttt cttactgagc actaggcaaa atgtagaagg ttcatatgac 3780

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aagaaacaca cagctcattt ctcaaaaaaa ggggaggaag aaaacttgga aggcttggga 3900

aatcaaacca agcaaattgt agagaaatat gcatgcacca caaggatatc tcctaataca 3960

agccagcaga attttgtcac gcaacgtagt aagagagctt tgaaacaatt cagactccca 4020

ctagaagaaa cagaacttga aaaaaggata attgtggatg acacctcaac ccagtggtcc 4080

aaaaacatga aacatttgac cccgagcacc ctcacacaga tagactacaa tgagaaggag 4140

aaaggggcca ttactcagtc tcccttatca gattgcctta cgaggagtca tagcatccct 4200

caagcaaata gatctccatt acccattgca aaggtatcat catttccatc tattagacct 4260

atatatctga ccagggtcct attccaagac aactctttct atcttccagc agcatcttat 4320

agaaagaaag attctggggt ccaagaaagc agtcatttct tacaaggagc caaaaaaaat 4380

aacctttctt tagccattct aaccttggag atgactggtg atcaaagaga ggttggctcc 4440

ctggggacaa gtgccacaaa ttcagtcaca tacaagaaag ttgagaacac tgttctcccg 4500

aaaccagact tgcccaaaac atctggcaaa gttgaattgc ttccaaaagt tcacatttat 4560

cagaaggacc tattccctac ggaaactagc aatgggtctc ctggccatct ggatctcgtg 4620

gaagggagcc ttcttcaggg aacagaggga gcgattaagt ggaatgaagc aaacagacct 4680

ggaaaagttc cctttctgag agtagcaaca gaaagctctg caaagactcc ctccaagcta 4740

ttggatcctc ttgcttggga taaccactat ggtactcaga taccaaaaga agagtggaaa 4800

tcccaagaga agtcaccaga aaaaacagct tttaagaaaa aggataccat tttgtccctg 4860

aacgcttgtg aaagcaatca tgcaatagca gcaataaatg agggacaaaa taagcccgaa 4920

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Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
    15                  20                  25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
30                  35                  40                  45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
                50                  55                  60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
            65                  70                  75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
        80                  85                  90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
    95                  100                 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110                 115                 120                 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
                130                 135                 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
            145                 150                 155

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
        160                 165                 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
    175                 180                 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190                 195                 200                 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
                210                 215                 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
            225                 230                 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
        240                 245                 250

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
    255                 260                 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270                 275                 280                 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
                290                 295                 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
            305                 310                 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
        320                 325                 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
    335                 340                 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350                 355                 360                 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
                370                 375                 380
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Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
            385                 390                 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
        400                 405                 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    415                 420                 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430                 435                 440                 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
                450                 455                 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
            465                 470                 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
        480                 485                 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    495                 500                 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510                 515                 520                 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
                530                 535                 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
            545                 550                 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
        560                 565                 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
    575                 580                 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590                 595                 600                 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
                610                 615                 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
            625                 630                 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
        640                 645                 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
    655                 660                 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670                 675                 680                 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
                690                 695                 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
            705                 710                 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
        720                 725                 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro
    735                 740                 745

Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp
750                 755                 760                 765

Ile Glu Lys Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys
                770                 775                 780

Ile Gln Asn Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser
            785                 790                 795

Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr
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        800                 805                 810

Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn
    815                 820                 825

Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly
830                 835                 840                 845

Asp Met Val Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu
                850                 855                 860

Lys Leu Gly Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys
            865                 870                 875

Val Ser Ser Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn
        880                 885                 890

Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met
    895                 900                 905

Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys
910                 915                 920                 925

Ser Ser Pro Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu
                930                 935                 940

Asn Asn Asp Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu
            945                 950                 955

Ser Ser Trp Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe
        960                 965                 970

Lys Gly Lys Arg Ala His Gly Pro Ala Leu Leu Thr Lys Asp Asn Ala
    975                 980                 985

Leu Phe Lys Val Ser Ile Ser Leu Leu Lys Thr  Asn Lys Thr Ser Asn
990                 995                 1000                1005

Asn Ser Ala Thr Asn  Arg Lys Thr His Ile  Asp Gly Pro Ser Leu
                1010                1015                1020

Leu Ile Glu Asn Ser  Pro Ser Val Trp Gln  Asn Ile Leu Glu Ser
                1025                1030                1035

Asp Thr Glu Phe Lys  Lys Val Thr Pro Leu  Ile His Asp Arg Met
                1040                1045                1050

Leu Met Asp Lys Asn  Ala Thr Ala Leu Arg  Leu Asn His Met Ser
                1055                1060                1065

Asn Lys Thr Thr Ser  Ser Lys Asn Met Glu  Met Val Gln Gln Lys
                1070                1075                1080

Lys Glu Gly Pro Ile  Pro Pro Asp Ala Gln  Asn Pro Asp Met Ser
                1085                1090                1095

Phe Phe Lys Met Leu  Phe Leu Pro Glu Ser  Ala Arg Trp Ile Gln
                1100                1105                1110

Arg Thr His Gly Lys  Asn Ser Leu Asn Ser  Gly Gln Gly Pro Ser
                1115                1120                1125

Pro Lys Gln Leu Val  Ser Leu Gly Pro Glu  Lys Ser Val Glu Gly
                1130                1135                1140

Gln Asn Phe Leu Ser  Glu Lys Asn Lys Val  Val Val Gly Lys Gly
                1145                1150                1155

Glu Phe Thr Lys Asp  Val Gly Leu Lys Glu  Met Val Phe Pro Ser
                1160                1165                1170

Ser Arg Asn Leu Phe  Leu Thr Asn Leu Asp  Asn Leu His Glu Asn
                1175                1180                1185

Asn Thr His Asn Gln  Glu Lys Lys Ile Gln  Glu Glu Ile Glu Lys
                1190                1195                1200

Lys Glu Thr Leu Ile  Gln Glu Asn Val Val  Leu Pro Gln Ile His
                1205                1210                1215
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Thr Val Thr Gly Thr  Lys Asn Phe Met Lys  Asn Leu Phe Leu Leu
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Ser Thr Arg Gln Asn  Val Glu Gly Ser Tyr  Asp Gly Ala Tyr Ala
                1235                1240                1245

Pro Val Leu Gln Asp  Phe Arg Ser Leu Asn  Asp Ser Thr Asn Arg
                1250                1255                1260

Thr Lys Lys His Thr  Ala His Phe Ser Lys  Lys Gly Glu Glu Glu
                1265                1270                1275

Asn Leu Glu Gly Leu  Gly Asn Gln Thr Lys  Gln Ile Val Glu Lys
                1280                1285                1290

Tyr Ala Cys Thr Thr  Arg Ile Ser Pro Asn  Thr Ser Gln Gln Asn
                1295                1300                1305

Phe Val Thr Gln Arg  Ser Lys Arg Ala Leu  Lys Gln Phe Arg Leu
                1310                1315                1320

Pro Leu Glu Glu Thr  Glu Leu Glu Lys Arg  Ile Ile Val Asp Asp
                1325                1330                1335

Thr Ser Thr Gln Trp  Ser Lys Asn Met Lys  His Leu Thr Pro Ser
                1340                1345                1350

Thr Leu Thr Gln Ile  Asp Tyr Asn Glu Lys  Glu Lys Gly Ala Ile
                1355                1360                1365

Thr Gln Ser Pro Leu  Ser Asp Cys Leu Thr  Arg Ser His Ser Ile
                1370                1375                1380

Pro Gln Ala Asn Arg  Ser Pro Leu Pro Ile  Ala Lys Val Ser Ser
                1385                1390                1395

Phe Pro Ser Ile Arg  Pro Ile Tyr Leu Thr  Arg Val Leu Phe Gln
                1400                1405                1410

Asp Asn Ser Ser His  Leu Pro Ala Ala Ser  Tyr Arg Lys Lys Asp
                1415                1420                1425

Ser Gly Val Gln Glu  Ser Ser His Phe Leu  Gln Gly Ala Lys Lys
                1430                1435                1440

Asn Asn Leu Ser Leu  Ala Ile Leu Thr Leu  Glu Met Thr Gly Asp
                1445                1450                1455

Gln Arg Glu Val Gly  Ser Leu Gly Thr Ser  Ala Thr Asn Ser Val
                1460                1465                1470

Thr Tyr Lys Lys Val  Glu Asn Thr Val Leu  Pro Lys Pro Asp Leu
                1475                1480                1485

Pro Lys Thr Ser Gly  Lys Val Glu Leu Leu  Pro Lys Val His Ile
                1490                1495                1500

Tyr Gln Lys Asp Leu  Phe Pro Thr Glu Thr  Ser Asn Gly Ser Pro
                1505                1510                1515

Gly His Leu Asp Leu  Val Glu Gly Ser Leu  Leu Gln Gly Thr Glu
                1520                1525                1530

Gly Ala Ile Lys Trp  Asn Glu Ala Asn Arg  Pro Gly Lys Val Pro
                1535                1540                1545

Phe Leu Arg Val Ala  Thr Glu Ser Ser Ala  Lys Thr Pro Ser Lys
                1550                1555                1560

Leu Leu Asp Pro Leu  Ala Trp Asp Asn His  Tyr Gly Thr Gln Ile
                1565                1570                1575

Pro Lys Glu Glu Trp  Lys Ser Gln Glu Lys  Ser Pro Glu Lys Thr
                1580                1585                1590

Ala Phe Lys Lys Lys  Asp Thr Ile Leu Ser  Leu Asn Ala Cys Glu
                1595                1600                1605
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Ser Asn His Ala Ile  Ala Ala Ile Asn Glu  Gly Gln Asn Lys Pro
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Glu Ile Glu Val Thr  Trp Ala Lys Gln Gly  Arg Thr Glu Arg Leu
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Cys Ser Gln Asn Pro  Pro Val Leu Lys Arg  His Gln Arg Glu Ile
                1640                1645                1650

Thr Arg Thr Thr Leu  Gln Ser Asp Gln Glu  Glu Ile Asp Tyr Asp
                1655                1660                1665

Asp Thr Ile Ser Val  Glu Met Lys Lys Glu  Asp Phe Asp Ile Tyr
                1670                1675                1680

Asp Glu Asp Glu Asn  Gln Ser Pro Arg Ser  Phe Gln Lys Lys Thr
                1685                1690                1695

Arg His Tyr Phe Ile  Ala Ala Val Glu Arg  Leu Trp Asp Tyr Gly
                1700                1705                1710

Met Ser Ser Ser Pro  His Val Leu Arg Asn  Arg Ala Gln Ser Gly
                1715                1720                1725

Ser Val Pro Gln Phe  Lys Lys Val Val Phe  Gln Glu Phe Thr Asp
                1730                1735                1740

Gly Ser Phe Thr Gln  Pro Leu Tyr Arg Gly  Glu Leu Asn Glu His
                1745                1750                1755

Leu Gly Leu Leu Gly  Pro Tyr Ile Arg Ala  Glu Val Glu Asp Asn
                1760                1765                1770

Ile Met Val Thr Phe  Arg Asn Gln Ala Ser  Arg Pro Tyr Ser Phe
                1775                1780                1785

Tyr Ser Ser Leu Ile  Ser Tyr Glu Glu Asp  Gln Arg Gln Gly Ala
                1790                1795                1800

Glu Pro Arg Lys Asn  Phe Val Lys Pro Asn  Glu Thr Lys Thr Tyr
                1805                1810                1815

Phe Trp Lys Val Gln  His His Met Ala Pro  Thr Lys Asp Glu Phe
                1820                1825                1830

Asp Cys Lys Ala Trp  Ala Tyr Phe Ser Asp  Val Asp Leu Glu Lys
                1835                1840                1845

Asp Val His Ser Gly  Leu Ile Gly Pro Leu  Leu Val Cys His Thr
                1850                1855                1860

Asn Thr Leu Asn Pro  Ala His Gly Arg Gln  Val Thr Val Gln Glu
                1865                1870                1875

Phe Ala Leu Phe Phe  Thr Ile Phe Asp Glu  Thr Lys Ser Trp Tyr
                1880                1885                1890

Phe Thr Glu Asn Met  Glu Arg Asn Cys Arg  Ala Pro Cys Asn Ile
                1895                1900                1905

Gln Met Glu Asp Pro  Thr Phe Lys Glu Asn  Tyr Arg Phe His Ala
                1910                1915                1920

Ile Asn Gly Tyr Ile  Met Asp Thr Leu Pro  Gly Leu Val Met Ala
                1925                1930                1935

Gln Asp Gln Arg Ile  Arg Trp Tyr Leu Leu  Ser Met Gly Ser Asn
                1940                1945                1950

Glu Asn Ile His Ser  Ile His Phe Ser Gly  His Val Phe Thr Val
                1955                1960                1965

Arg Lys Lys Glu Glu  Tyr Lys Met Ala Leu  Tyr Asn Leu Tyr Pro
                1970                1975                1980

Gly Val Phe Glu Thr  Val Glu Met Leu Pro  Ser Lys Ala Gly Ile
                1985                1990                1995

Trp Arg Val Glu Cys  Leu Ile Gly Glu His  Leu His Ala Gly Met
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Ser Thr Leu Phe Leu  Val Tyr Ser Asn Lys  Cys Gln Thr Pro Leu
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Gly Met Ala Ser Gly  His Ile Arg Asp Phe  Gln Ile Thr Ala Ser
                2030                2035                2040

Gly Gln Tyr Gly Gln  Trp Ala Pro Lys Leu  Ala Arg Leu His Tyr
                2045                2050                2055

Ser Gly Ser Ile Asn  Ala Trp Ser Thr Lys  Glu Pro Phe Ser Trp
                2060                2065                2070

Ile Lys Val Asp Leu  Leu Ala Pro Met Ile  Ile His Gly Ile Lys
                2075                2080                2085

Thr Gln Gly Ala Arg  Gln Lys Phe Ser Ser  Leu Tyr Ile Ser Gln
                2090                2095                2100

Phe Ile Ile Met Tyr  Ser Leu Asp Gly Lys  Lys Trp Gln Thr Tyr
                2105                2110                2115

Arg Gly Asn Ser Thr  Gly Thr Leu Met Val  Phe Phe Gly Asn Val
                2120                2125                2130

Asp Ser Ser Gly Ile  Lys His Asn Ile Phe  Asn Pro Pro Ile Ile
                2135                2140                2145

Ala Arg Tyr Ile Arg  Leu His Pro Thr His  Tyr Ser Ile Arg Ser
                2150                2155                2160

Thr Leu Arg Met Glu  Leu Met Gly Cys Asp  Leu Asn Ser Cys Ser
                2165                2170                2175

Met Pro Leu Gly Met  Glu Ser Lys Ala Ile  Ser Asp Ala Gln Ile
                2180                2185                2190

Thr Ala Ser Ser Tyr  Phe Thr Asn Met Phe  Ala Thr Trp Ser Pro
                2195                2200                2205

Ser Lys Ala Arg Leu  His Leu Gln Gly Arg  Ser Asn Ala Trp Arg
                2210                2215                2220

Pro Gln Val Asn Asn  Pro Lys Glu Trp Leu  Gln Val Asp Phe Gln
                2225                2230                2235

Lys Thr Met Lys Val  Thr Gly Val Thr Thr  Gln Gly Val Lys Ser
                2240                2245                2250

Leu Leu Thr Ser Met  Tyr Val Lys Glu Phe  Leu Ile Ser Ser Ser
                2255                2260                2265

Gln Asp Gly His Gln  Trp Thr Leu Phe Phe  Gln Asn Gly Lys Val
                2270                2275                2280

Lys Val Phe Gln Gly  Asn Gln Asp Ser Phe  Thr Pro Val Val Asn
                2285                2290                2295

Ser Leu Asp Pro Pro  Leu Leu Thr Arg Tyr  Leu Arg Ile His Pro
                2300                2305                2310

Gln Ser Trp Val His  Gln Ile Ala Leu Arg  Met Glu Val Leu Gly
                2315                2320                2325

Cys Glu Ala Gln Asp  Leu Tyr Asp Lys Thr  His Thr Cys Pro Pro
                2330                2335                2340

Cys Pro Ala Pro Glu  Leu Leu Gly Gly Pro  Ser Val Phe Leu Phe
                2345                2350                2355

Pro Pro Lys Pro Lys  Asp Thr Leu Met Ile  Ser Arg Thr Pro Glu
                2360                2365                2370

Val Thr Cys Val Val  Val Asp Val Ser His  Glu Asp Pro Glu Val
                2375                2380                2385

Lys Phe Asn Trp Tyr  Val Asp Gly Val Glu  Val His Asn Ala Lys
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Thr Lys Pro Arg Glu  Glu Gln Tyr Asn Ser  Thr Tyr Arg Val Val
                2405                2410                2415

Ser Val Leu Thr Val  Leu His Gln Asp Trp  Leu Asn Gly Lys Glu
                2420                2425                2430

Tyr Lys Cys Lys Val  Ser Asn Lys Ala Leu  Pro Ala Pro Ile Glu
                2435                2440                2445

Lys Thr Ile Ser Lys  Ala Lys Gly Gln Pro  Arg Glu Pro Gln Val
                2450                2455                2460

Tyr Thr Leu Pro Pro  Ser Arg Asp Glu Leu  Thr Lys Asn Gln Val
                2465                2470                2475

Ser Leu Thr Cys Leu  Val Lys Gly Phe Tyr  Pro Ser Asp Ile Ala
                2480                2485                2490

Val Glu Trp Glu Ser  Asn Gly Gln Pro Glu  Asn Asn Tyr Lys Thr
                2495                2500                2505

Thr Pro Pro Val Leu  Asp Ser Asp Gly Ser  Phe Phe Leu Tyr Ser
                2510                2515                2520

Lys Leu Thr Val Asp  Lys Ser Arg Trp Gln  Gln Gly Asn Val Phe
                2525                2530                2535

Ser Cys Ser Val Met  His Glu Ala Leu His  Asn His Tyr Thr Gln
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Lys Ser Leu Ser Leu  Ser Pro Gly Lys
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gggagtctgg ccaaggaaaa gacacagacc ttgcacaaat ttatactact ttttgctgta    660

tttgatgaag ggaaaagttg gcactcagaa acaaagaact ccttgatgca ggatagggat    720

gctgcatctg ctcgggcctg gcctaaaatg cacacagtca atggttatgt aaacaggtct    780

ctgccaggtc tgattggatg ccacaggaaa tcagtctatt ggcatgtgat tggaatgggc    840
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accactcctg aagtgcactc aatattcctc gaaggtcaca catttcttgt gaggaaccat 900

cgccaggcgt ccttggaaat ctcgccaata actttcctta ctgctcaaac actcttgatg 960

gaccttggac agtttctact gttttgtcat atctcttccc accaacatga tggcatggaa 1020

gcttatgtca aagtagacag ctgtccagag gaaccccaac tacgaatgaa aaataatgaa 1080

gaagcggaag actatgatga tgatcttact gattctgaaa tggatgtggt caggtttgat 1140

gatgacaact ctccttcctt tatccaaatt cgctcagttg ccaagaagca tcctaaaact 1200

tgggtacatt acattgctgc tgaagaggag gactgggact atgctccctt agtcctcgcc 1260

cccgatgaca gaagttataa aagtcaatat ttgaacaatg gccctcagcg gattggtagg 1320

aagtacaaaa aagtccgatt tatggcatac acagatgaaa cctttaagac tcgtgaagct 1380

attcagcatg aatcaggaat cttgggacct ttactttatg gggaagttgg agacacactg 1440

ttgattatat ttaagaatca agcaagcaga ccatataaca tctaccctca cggaatcact 1500

gatgtccgtc ctttgtattc aaggagatta ccaaaaggtg taaaacattt gaaggatttt 1560

ccaattctgc caggagaaat attcaaatat aaatggacag tgactgtaga agatgggcca 1620

actaaatcag atcctcggtg cctgacccgc tattactcta gtttcgttaa tatggagaga 1680

gatctagctt caggactcat tggccctctc ctcatctgct acaaagaatc tgtagatcaa 1740

agaggaaacc agataatgtc agacaagagg aatgtcatcc tgttttctgt atttgatgag 1800

aaccgaagct ggtacctcac agagaatata caacgctttc tccccaatcc agctggagtg 1860

cagcttgagg atccagagtt ccaagcctcc aacatcatgc acagcatcaa tggctatgtt 1920

tttgatagtt tgcagttgtc agtttgtttg catgaggtgg catactggta cattctaagc 1980

attggagcac agactgactt cctttctgtc ttcttctctg gatatacctt caaacacaaa 2040

atggtctatg aagacacact caccctattc ccattctcag gagaaactgt cttcatgtcg 2100

atggaaaacc caggtctatg gattctgggg tgccacaact cagactttcg gaacagaggc 2160

atgaccgcct tactgaaggt ttctagttgt gacaagaaca ctggtgatta ttacgaggac 2220

agttatgaag atatttcagc atacttgctg agtaaaaaca atgccattga accaagagac 2280

aaaactcaca catgcccacc gtgcccagct ccagaactcc tgggcggacc gtcagtcttc 2340

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc 2400

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 2460

gtggaggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 2520

gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 2580

aaggtctcca acaaagccct cccagccccc atcgagaaaa ccatctccaa agccaaaggg 2640

cagccccgag aaccacaggt gtacaccctg cccccatccc gggatgagct gaccaagaac 2700

caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 2760

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgtt ggactccgac 2820

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 2880

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 2940

tccctgtctc cgggtaaa 2958

<210> SEQ ID NO 8
<211> LENGTH: 986
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HC-Fc

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<220> FEATURE:
<221> NAME/KEY: SIGNAL
<222> LOCATION: (1)..(19)
<220> FEATURE:
<221> NAME/KEY: mat_peptide
<222> LOCATION: (20)..(986)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (760)..(986)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 8

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Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
                -15                 -10                 -5

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
        -1  1               5                   10

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
    15                  20                  25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
30                  35                  40                  45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
                50                  55                  60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
            65                  70                  75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
        80                  85                  90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
    95                  100                 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110                 115                 120                 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
                130                 135                 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
            145                 150                 155

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
        160                 165                 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
    175                 180                 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190                 195                 200                 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
                210                 215                 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
            225                 230                 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
        240                 245                 250

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
    255                 260                 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270                 275                 280                 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
                290                 295                 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
            305                 310                 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
        320                 325                 330
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Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
    335                 340                 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350                 355                 360                 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
                370                 375                 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
            385                 390                 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
        400                 405                 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    415                 420                 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430                 435                 440                 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
                450                 455                 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
            465                 470                 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
        480                 485                 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    495                 500                 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510                 515                 520                 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
                530                 535                 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
            545                 550                 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
        560                 565                 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
    575                 580                 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590                 595                 600                 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
                610                 615                 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
            625                 630                 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
        640                 645                 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
    655                 660                 665

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670                 675                 680                 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
                690                 695                 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
            705                 710                 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
        720                 725                 730

Asn Asn Ala Ile Glu Pro Arg Asp Lys Thr His Thr Cys Pro Pro Cys
    735                 740                 745

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
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750                 755                 760                 765

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                770                 775                 780

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
            785                 790                 795

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        800                 805                 810

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    815                 820                 825

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
830                 835                 840                 845

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                850                 855                 860

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
            865                 870                 875

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        880                 885                 890

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    895                 900                 905

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
910                 915                 920                 925

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                930                 935                 940

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            945                 950                 955

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        960                 965


<210> SEQ ID NO 9
<211> LENGTH: 2973
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Heavy Chain (HC)-Fc (5 amino acid linker
      between HC and Fc)
<220> FEATURE:
<221> NAME/KEY: misc_signal
<222> LOCATION: (1)..(57)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2278)..(2292)
<223> OTHER INFORMATION: 5 amino acid linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2293)..(2973)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 9

atgcaaatag agctctccac ctgcttcttt ctgtgccttt tgcgattctg ctttagtgcc     60

accagaagat actacctggg tgcagtggaa ctgtcatggg actatatgca aagtgatctc    120

ggtgagctgc ctgtggacgc aagatttcct cctagagtgc caaaatcttt tccattcaac    180

acctcagtcg tgtacaaaaa gactctgttt gtagaattca cggatcacct tttcaacatc    240

gctaagccaa ggccaccctg gatgggtctg ctaggtccta ccatccaggc tgaggtttat    300

gatacagtgg tcattacact taagaacatg gcttcccatc ctgtcagtct tcatgctgtt    360

ggtgtatcct actggaaagc ttctgaggga gctgaatatg atgatcagac cagtcaaagg    420

gagaaagaag atgataaagt cttccctggt ggaagccata catatgtctg gcaggtcctg    480
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aaagagaatg gtccaatggc ctctgaccca ctgtgcctta cctactcata tctttctcat 540

gtggacctgg taaaagactt gaattcaggc ctcattggag ccctactagt atgtagagaa 600

gggagtctgg ccaaggaaaa gacacagacc ttgcacaaat ttatactact ttttgctgta 660

tttgatgaag ggaaaagttg gcactcagaa acaaagaact ccttgatgca ggatagggat 720

gctgcatctg ctcgggcctg gcctaaaatg cacacagtca atggttatgt aaacaggtct 780

ctgccaggtc tgattggatg ccacaggaaa tcagtctatt ggcatgtgat tggaatgggc 840

accactcctg aagtgcactc aatattcctc gaaggtcaca catttcttgt gaggaaccat 900

cgccaggcgt ccttggaaat ctcgccaata actttcctta ctgctcaaac actcttgatg 960

gaccttggac agtttctact gttttgtcat atctcttccc accaacatga tggcatggaa 1020

gcttatgtca aagtagacag ctgtccagag gaaccccaac tacgaatgaa aaataatgaa 1080

gaagcggaag actatgatga tgatcttact gattctgaaa tggatgtggt caggtttgat 1140

gatgacaact ctccttcctt tatccaaatt cgctcagttg ccaagaagca tcctaaaact 1200

tgggtacatt acattgctgc tgaagaggag gactgggact atgctccctt agtcctcgcc 1260

cccgatgaca gaagttataa aagtcaatat ttgaacaatg gccctcagcg gattggtagg 1320

aagtacaaaa aagtccgatt tatggcatac acagatgaaa cctttaagac tcgtgaagct 1380

attcagcatg aatcaggaat cttgggacct ttactttatg gggaagttgg agacacactg 1440

ttgattatat ttaagaatca agcaagcaga ccatataaca tctaccctca cggaatcact 1500

gatgtccgtc ctttgtattc aaggagatta ccaaaaggtg taaaacattt gaaggatttt 1560

ccaattctgc caggagaaat attcaaatat aaatggacag tgactgtaga agatgggcca 1620

actaaatcag atcctcggtg cctgacccgc tattactcta gtttcgttaa tatggagaga 1680

gatctagctt caggactcat tggccctctc ctcatctgct acaaagaatc tgtagatcaa 1740

agaggaaacc agataatgtc agacaagagg aatgtcatcc tgttttctgt atttgatgag 1800

aaccgaagct ggtacctcac agagaatata caacgctttc tccccaatcc agctggagtg 1860

cagcttgagg atccagagtt ccaagcctcc aacatcatgc acagcatcaa tggctatgtt 1920

tttgatagtt tgcagttgtc agtttgtttg catgaggtgg catactggta cattctaagc 1980

attggagcac agactgactt cctttctgtc ttcttctctg gatatacctt caaacacaaa 2040

atggtctatg aagacacact caccctattc ccattctcag gagaaactgt cttcatgtcg 2100

atggaaaacc caggtctatg gattctgggg tgccacaact cagactttcg gaacagaggc 2160

atgaccgcct tactgaaggt ttctagttgt gacaagaaca ctggtgatta ttacgaggac 2220

agttatgaag atatttcagc atacttgctg agtaaaaaca atgccattga accaagaagc 2280

ttctcccaga atgacaaaac tcacacatgc ccaccgtgcc cagctccaga actcctgggc 2340

ggaccgtcag tcttcctctt ccccccaaaa cccaaggaca ccctcatgat ctcccggacc 2400

cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 2460

tggtacgtgg acggcgtgga ggtgcataat gccaagacaa agccgcggga ggagcagtac 2520

aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 2580

aaggagtaca agtgcaaggt ctccaacaaa gccctcccag cccccatcga gaaaaccatc 2640

tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgccccc atcccgggat 2700

gagctgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta tcccagcgac 2760

atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cacgcctccc 2820

gtgttggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 2880

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acgcagaaga gcctctccct gtctccgggt aaa 2973

<210> SEQ ID NO 10
<211> LENGTH: 991
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HC+5-Fc
<220> FEATURE:
<221> NAME/KEY: SIGNAL
<222> LOCATION: (1)..(19)
<220> FEATURE:
<221> NAME/KEY: mat_peptide
<222> LOCATION: (20)..(991)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (760)..(764)
<223> OTHER INFORMATION: B domain
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (765)..(991)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 10

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
-15 -10 -5

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
-1 1 5 10

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
15 20 25

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
30 35 40 45

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
50 55 60

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
65 70 75

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
80 85 90

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
95 100 105

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
110 115 120 125

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
130 135 140

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
145 150 155

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
160 165 170

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
175 180 185

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
190 195 200 205

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
210 215 220

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
225 230 235

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
240 245 250

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Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
    255                 260                 265

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
270                 275                 280                 285

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
                290                 295                 300

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
            305                 310                 315

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
        320                 325                 330

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
    335                 340                 345

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
350                 355                 360                 365

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
                370                 375                 380

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
            385                 390                 395

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
        400                 405                 410

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    415                 420                 425

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
430                 435                 440                 445

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
                450                 455                 460

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
            465                 470                 475

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
        480                 485                 490

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    495                 500                 505

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
510                 515                 520                 525

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
                530                 535                 540

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
            545                 550                 555

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
        560                 565                 570

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
    575                 580                 585

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
590                 595                 600                 605

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
                610                 615                 620

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
            625                 630                 635

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
        640                 645                 650

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
    655                 660                 665
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Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
670                 675                 680                 685

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
                690                 695                 700

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
            705                 710                 715

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
        720                 725                 730

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Asp Lys Thr His
    735                 740                 745

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
750                 755                 760                 765

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                770                 775                 780

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            785                 790                 795

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        800                 805                 810

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    815                 820                 825

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
830                 835                 840                 845

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                850                 855                 860

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            865                 870                 875

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        880                 885                 890

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    895                 900                 905

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
910                 915                 920                 925

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                930                 935                 940

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            945                 950                 955

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        960                 965                 970


<210> SEQ ID NO 11
<211> LENGTH: 2793
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Light Chain (LC)-Fc
<220> FEATURE:
<221> NAME/KEY: misc_signal
<222> LOCATION: (1)..(60)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2113)..(2793)
<223> OTHER INFORMATION: Fc region

<400> SEQUENCE: 11

atggagacag acacactcct gctatgggta ctgctgctct gggttccagg ttccactggt     60

gaaataactc gtactactct tcagtcagat caagaggaaa ttgactatga tgataccata    120

tcagttgaaa tgaagaagga agattttgac atttatgatg aggatgaaaa tcagagcccc    180
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cgcagctttc aaaagaaaac acgacactat tttattgctg cagtggagag gctctgggat 240

tatgggatga gtagctcccc acatgttcta agaaacaggg ctcagagtgg cagtgtccct 300

cagttcaaga aagttgtttt ccaggaattt actgatggct cctttactca gcccttatac 360

cgtggagaac taaatgaaca tttgggactc ctggggccat atataagagc agaagttgaa 420

gataatatca tggtaacttt cagaaatcag gcctctcgtc cctattcctt ctattctagc 480

cttatttctt atgaggaaga tcagaggcaa ggagcagaac ctagaaaaaa ctttgtcaag 540

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Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp
        15                  20                  25

Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln
    30                  35                  40

Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp
45                  50                  55                  60

Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser
                65                  70                  75

Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp
            80                  85                  90

Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu
        95                  100                 105

Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met
    110                 115                 120

Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser
125                 130                 135                 140

Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys
                145                 150                 155

Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln
            160                 165                 170

His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala
        175                 180                 185

Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile
    190                 195                 200

Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly
205                 210                 215                 220

Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp
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Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg
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Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr
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Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly
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Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His Val Phe Thr
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Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro
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Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp
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Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr
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Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala
365                 370                 375                 380

Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly
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Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn
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Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu
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Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys
    430                 435                 440

Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp
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Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met
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Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe
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Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn
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Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala
525                 530                 535                 540

Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser
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Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg
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Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys
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Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu
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605                 610                 615                 620

His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln
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Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro
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Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
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Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
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His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    750                 755                 760

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
765                 770                 775                 780

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                785                 790                 795

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
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Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        815                 820                 825

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    830                 835                 840

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
845                 850                 855                 860

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                865                 870                 875

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            880                 885                 890

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        895                 900                 905

Pro Gly Lys
    910
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What is claimed is:

1. A method for reducing the incidence of a bleeding episode in a human subject in need thereof comprising administering to the subject multiple doses of a long-acting Factor VIII (FVIII) polypeptide at a dosing interval of 3 to 7 days between two doses of the long-acting FVIII polypeptide,

wherein the therapeutic dose is about 25 IU/kg to about 65 IU/kg,

wherein the long-acting FVIII polypeptide is an FVIIIFc monomer dimer hybrid-comprising a FVIII portion and two Fc portions, wherein one of the Fc portions is fused to the C-terminus of the light chain of the FVIII portion.

2. The method of claim **1**, wherein the subject has hemophilia A.

3. The method of claim **1**, wherein the reducing the incidence of a bleeding episode prevents or treats the bleeding episode.

4. The method of claim **1**, wherein the administration is for prophylaxis of the bleeding episode or for tailored prophylaxis.

5. The method of claim **1**, wherein a trough level of plasma Factor VIII:C in the subject after the administration is maintained above 1 IU/dl.

6. The method of claim **1**, wherein the therapeutic dose is 25 IU/kg, 30 IU/kg, 35 IU/kg, 40 IU/kg, 45 IU/kg, 50 IU/kg, 55 IU/kg, 60 IU/kg, 65 UI/kg.

7. The method of claim **1**, wherein the dosing interval is three days, four days, five days, six days, or seven days.

8. The method of claim **1**, wherein the dosing interval is about twice a week.

9. The method of claim **1**, wherein the therapeutic dose is about 65 IU/kg.

10. The method of claim **9**, wherein the dosing interval is about four days.

11. The method of claim **1**, comprising administering to the subject twice weekly, a first therapeutic dose of about 25 IU/kg to about 65 IU/kg of the long-acting FVIII polypeptide and a second therapeutic dose of about 25 IU/kg to about 65 IU/kg of the long-acting FVIII polypeptide.

12. The method of claim **11**, wherein the dosing interval between the first dose and the second dose is 72 hours to five days.

13. The method of claim **1**, wherein the administration resolves greater than 5-20%, greater than 5-15%, greater than 5-10%, greater than 10-20%, or greater than 10-15% of bleeding episode.

14. The method of claim **1**, wherein the long-acting FVIII polypeptide is pegylated Factor VIII.

15. The method of claim 1, wherein the FVIII portion comprises LH-length factor VIII, mature factor VIII, or factor VIII with a full or partial deletion of the B domain.

16. The method of claim 1, which further exhibits one or more characteristics selected from:

(i) wherein a mean clearance (CL) (activity) in the subject is about 2.33±1.08 mL/hour/kg or less;

(ii) wherein a mean residence time (MRT) (activity) in the subject is about 1.5 fold longer than the MRT of a polypeptide consisting of said FVIII portion;

(iii) wherein a $T_{1/2}$ (activity) in the subject is about 1.5 fold longer than the mean $T_{1/2}$ (activity) of a polypeptide consisting of said FVIII portion;

(iv) wherein a mean incremental recovery (K value) in the subject is about 90% of the incremental recovery of a polypeptide consisting of said FVIII portion;

(v) wherein a mean Vss (activity) in the subject is about 37.7 to 79.4 mL/kg;

(vi) wherein a mean AUC/dose (activity) in the subject is about 19.2*h/dL per IU/kg to 81.7 IU*h/dL per IU/kg; and

(vii) a combination thereof.

17. The method of claim 1, wherein the therapeutic dose is about 50 IU/kg or about 65 IU/kg.

18. The method of claim 11, wherein the first dose is about 50 IU/kg and the second dose is about 50 IU/kg.

19. The method of claim 18, wherein the second dose is administered three days or four days after the first dose.

20. The method of claim 11, wherein the first dose is about 65 IU/kg and the second dose is about 65 IU/kg.

21. The method of claim 20, wherein the second dose is administered five days or one week after administration of the first dose.

22. The method of claim 19, wherein a trough level of plasma Factor VIII:C in the subject after the administration is maintained above 1 IU/dl.

23. The method of claim 21, wherein a trough level of plasma Factor VIII:C in the subject after the administration is maintained above 1 IU/dl.

24. A method for prophylactic treatment of a spontaneous bleeding episode in a human subject comprising administering to the subject multiple doses of a long-acting FVIII polypeptide at a dosing interval of about 3 to 7 days between two doses of the long-acting FVIII polypeptide,

wherein the therapeutic dose is about 25 IU/kg to 65 IU/kg, and

wherein the long-acting FVIII polypeptide is a FVIIIFc monomer dimer hybrid-comprising a FVIII portion and two Fc portions, wherein one of the Fc portions is fused to the C-terminus of the light chain of the FVIII portion, and

wherein the spontaneous bleeding episode is prophylactically treated.

25. The method of claim 24, wherein the FVIII portion comprises full-length factor VIII, mature factor VIII, or factor VIII with a full or partial deletion of the B domain.

26. A method for reducing the incidence of a bleeding episode in a human subject comprising administering to the subject multiple doses of a chimeric polypeptide comprising a FVIIIFc monomer dimer hybrid at a dosing interval of about 3 to 7 days between two doses of the chimeric polypeptide,

wherein the therapeutic dose is about 25 IU/Kg to about 65 IU/kg, and

wherein the FVIIIFc monomer dimer hybrid comprises a FVIII portion and two Fc portions, wherein one of the two Fc portions is fused to the C-terminus of the light chain of the FVIII portion, and

wherein an AUC/dose in the subject is about 19.2-81.7_IU*h/kL per IU/kg, a clearance (CL) (activity) in the subject is about 1.22-5.19 mL/hour/kg, or both.

27. The method of claim 1, wherein the FVIII portion comprises an amino acid sequence at least 95% identical to amino acids 1 to 1438 of SEQ ID NO: 2.

28. The method of claim 1, wherein the FVIII portion comprises amino acids 1 to 1438 of SEQ ID NO: 2.

29. The method of claim 1, wherein the long-acting FVIII polypeptide comprises an amino acid sequence at least 95% identical to amino acids 1 to 1665 of SEQ ID NO: 2.

30. The method of claim 1, wherein the long-acting FVIII polypeptide comprises amino acids 1 to 1665 of SEQ ID NO: 2.

31. The method of claim 24, wherein the FVIIIFc monomer dimer hybrid comprises amino acids 1 to 1665 of SEQ ID NO: 2 and amino acids 1 to 227 of SEQ ID NO: 4.

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